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BY

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INTRODUCTION

THE DICYEMID MESOZOA are small vermiform organisms that occur in great numbers attached to the renal organs of various cephalopod mollusks. Knowledge of the dicyemids is embodied in several works dealing primarily with species in Europe. Those in America are largely unknown, the only published accounts of them being two short preliminary papers by Wheeler (1897, 1899), two by McConnaughey (1938, 1941), and part of the recent monograph by Nouvel (1947). These works do not constitute an adequate survey of the group.

The present paper is confined to descriptions of the species from the coast of California and Lower California, and to discussion of certain questions relating to classification. Keys to all known species are included to facilitate determination and comparison. A detailed discussion of questions relating to the morphology and life cycle will be given in a later paper.

The writer is especially indebted to Dr. Harold Kirby and the late Dr. S. F. Light of the University of California, under whose direction the work was conducted; to Dr. Martin Johnson, Dr. Dennis L. Fox, Dr. Wesley R. Coe, Dr. Carl Hubbs, Dr. Henry Kritzler, and Mr. Sheldon C. Crane of the Scripps Institution of Oceanography, for aid and suggestions during the course of the study and help in the collection of cephalopods; and to Dr. S. S. Berry of Redlands, California, and Dr. Grace Pickford of Yale University for aid in the determination of cephalopods. Dr. Horace Stunkard kindly read the manuscript and offered numerous helpful suggestions.

MATERIALS AND METHODS

The descriptive studies were based primarily on cover-slip smear preparations from the kidneys of freshly killed cephalopods, fixed in Bouin's fluid and stained with Ehrlich's acid hematoxylin. Living specimens of most of the species have also been studied.

Cephalopods obtained at localities from San Francisco Bay, California, to Rosarito Beach, Lower California, and from the Coronado Islands have been examined for dicyemids. All octopuses taken along the California coast, except one very small one, were infected. The pelagic squid *Loligo opalescens* taken at two stations near La Jolla, California, one about three miles offshore just outside the kelp beds, the other about six miles offshore, showed no infections in a series of sixteen living specimens. A number of specimens of the squid obtained from fish markets in San Francisco and San Diego from time

* Contribution from the Scripps Institution of Oceanography (new series), number 424, and the Department of Zoölogy, University of California, Berkeley.

to time also contained no mesozoa. A very few dicyemids were present on some smear preparations kindly sent to the author by Mr. N. Riser of the Hopkins Marine Station, made from a specimen of *Loligo opalescens* taken in Monterey Bay in September, 1947. However, there was insufficient material present from which to make a specific diagnosis. This is the first record of infection of squids from the Pacific coast by mesozoa. A paper nautilus *Argonauta pacifica* from near Cedros Island was free from these parasites.

In addition, the writer has examined a series of sixteen living octopuses on Oahu Island, Territory of Hawaii, and one on Kwajalein in the Marshall Islands. None of the octopuses from the Central Pacific islands was found to be infected.

DESCRIPTION OF SPECIES FROM THE CALIFORNIA COAST

The dicyemids comprise a total of 31 known species, including 13 from the California coast. They are distributed among three genera as follows:

Genus	Total known species	California species
<i>Dicyemenea</i>	10	7
<i>Dicyema</i>	18	5
<i>Conocyema</i>	3	1

The descriptions and figures refer to the vermiform phases ordinarily seen, the primary and secondary nematogens and rhombogens, except where otherwise indicated. For those species which have not been found along the California coast a brief explanatory paragraph is added to the key, giving their distribution, hosts, and additional points helpful in determination.

Genus DICYEMENNEA Whitman, 1883

Diagnosis.—Calotte 9-celled; almost always orthotropical; propolar cells 4, 2 dorsal and 2 ventral; metapolar cells 5, 1 dorsal, 2 dorsolateral, 2 ventral; vermiform larvae of most species with abortive second axial cell located anterior to the axial cell proper, in the region of the metapolar cells; this second axial cell usually absorbed shortly after escape of larva from parent nematogen, leaving no trace in adult.

Type species.—*Dicyema eleddones* Wagener, 1857.

KEY TO THE SPECIES OF DICYEMENNEA

1. An abortive second axial cell present as a constant feature in inclosed vermiform larvae, just anterior to the axial cell proper.....2
No abortive second axial cell formed during development of vermiform larva.
A large species often 4 to 6 mm. long, with clear nongranular somatic cells, no verruciform cells; total number of somatic cells 23. Hosts: *Sepia officinalis* Linnaeus and *Sepia orbignyana* Férussac. Distribution:
Mediterranean.....*gracile* (Wagener, 1857)
2. (1) Axial cell ends anteriorly behind or between posterior ends of metapolar cells; calotte conical, extending forward beyond axial cell as a solid body; abortive second axial cell of vermiform larvae not usually farther forward than middle of metapolar cells; cephalic swelling often widest at level of parapolar cells or even farther back; cell number usually variable.....3
Axial cell extends forward to or between the bases of the propolar cells, calotte fitting over its anterior end in manner of cap or helmet, or taking form of flattened

- disc at more or less truncated end of body; abortive second axial cell of vermiform larvae well forward near base of propolar cells; cell number constant or variable within small limits 7
3. (2) Medium-sized to large species, adults usually exceeding 1 mm. in length; uropolar cells and sometimes 1 or more diapolars verruciform.....4
Small, adults usually less than 1 mm. in length; generally no verruciform cells; total number of somatic cells 21 to 26, commonly 25.....*abasi* n. sp.
4. (3) Total number of somatic cells 27 to 42, almost always in the thirties; adults mostly 1 to 4.5 mm. long.....6
Total number of somatic cells 23 to 29; adults usually 0.5 to 2.0 mm. long.....5
5. (4) Somatic cell number usually 24 to 27, sometimes exceeding these limits in either direction; uropolar cells verruciform, others occasionally so; cytoplasmic protein reserves formed as rather large irregularly shaped lightly staining aggregates in somatic cells of trunk, particularly the verruciform cells; diapolar cells of rhombogens sometimes heavily charged with small granules.....*abelis* n. sp.
Somatic cell number usually 23, sometimes higher; uropolar cells and commonly 1 or 2 diapolars verruciform; verruciform cells large, characterized by numerous deeply staining bodies in their cytoplasmic network.....*adscita* n. sp.
6. (4) Somatic cells, except verruciform cells, parapolars, and sometimes the anterior diapolar cells, quite clear in life or lightly granular; body long and slender; total number of somatic cells 27 to 42, almost always in the thirties
.....*californica* McConnaughey, 1941
Somatic cells, except those of calotte and verruciform cells commonly heavily charged with rounded proteinaceous refractile granules giving them an inflated dark appearance in life, axial cell forming a median transparent band; calotte narrower than anterior end of trunk, extending forward from it as a clear conical projection in sharp contrast to the dark heavily granular trunk; total number of somatic cells usually in the low thirties.....*granularis* n. sp.
7. (2) Calotte conical; infusoriform with only 1 free nucleus and 1 germ cell in each cell within the urn; somatic cell number 23.....8
Calotte flattened, discoid; propolar cells notably smaller than, and ringed by, the metapolar cells, the 2 groups together forming a flattened disk; infusoriform with 2 nuclei and 1 germ cell in each cell within the urn.....9
8. (7) Adults slender, often 5 to 8 mm. long.
A much elongated species, swollen at the anterior end, the parapolar cells subequal to the calotte and taking part in the cephalic swelling; propolar cells much smaller than the metapolars; no verruciform cells. Hosts: *Eledone cirrhosa* (Lamarck), *E. moschata* (Lamarck), and *Octopus saluasi* Vérany. Distribution: Mediterranean*eledones* (Wagener, 1857)
Adults robust, short, less than 2 mm. long.
A small stocky species without verruciform cells, generally widest anteriorly and tapering gradually to the posterior end; somatic cells typically 23, characterized by one or more falciform acidophile proteinaceous rods in each. Hosts: *Octopus vulgaris* Lamarck, and *Eledone cirrhosa* (Lamarck). Distribution: Atlantic coasts of Europe, Mediterranean.....*lameerei* Nouvel, 1932
9. (7) A strong constriction behind the parapolar cells setting off the cephalic swelling from the rest of the body; calotte deeply staining in contrast to the remaining somatic cells; metapolar cells inflated, commonly extending forward around and beyond the propolar cells making the calotte concave; adults usually less than 1 mm. long; total number of somatic cells constant, 25.....*abbreviata* n. sp.
No strong constriction behind the parapolar cells; calotte flattened but not concave, often obliquely tilted; contrast in staining between calotte and trunk much less marked; total number of somatic cells usually 27
.....*brevicephala* McConnaughey, 1941

Dicyemennea californica McConnaughey, 1941

(Pl. 1, fig. 1; pl. 2, figs. 19-22)

Diagnosis.—Adults usually 1 to 4 mm. long, slender, transparent in life except for the granular contents of the parapolar, anterior diapolar, and verruciform cells; total number of somatic cells variable, from 27 to 42, usually 30 to 39; tendency to segregate into 2 overlapping groups, one with 30 to 34, the other with 35 to 37 (higher numbers exceptional) somatic cells; cephalic swelling composed of the calotte, the parapolar, and the first 2 or 3 diapolar cells, generally broadest at the level of the parapolars; calotte conical, orthotropical; parapolar cells shield-shaped, about equal to the calotte in length or somewhat exceeding it; axial cell extends forward only a short distance between the bases of the metapolar cells, not reaching the propolars; uropolar cells verruciform, other verruciform cells occasionally present.

Vermiform larvae with abortive second axial cell at anterior end of the axial cell between the basal ends of the metapolars; calotte of larvae sharply pointed; larvae elongate, attaining 0.15 to 0.3 mm. before escaping from the axial cell of the parent nematogen.

Rhombogens often slightly longer and broader than nematogens; somatic cells of trunk in this phase commonly with 1 to 5 accessory nuclei of various sizes; axial cell with paranuclei corresponding in number to the number of infusorigens present.

Infusoriform 38 to 45 microns long, rounded posteriorly, each cell within the urn containing 2 nuclei and 1 germ cell.

Host.—*Octopus bimaculoides* Pickford and McConnaughey.

Distribution.—Southern and Lower California.

Types.—Syntypes USNM cat. no. 22682. Paratypes in USNM and in collections of author and of Nouvel.

McConnaughey (1941) stated that the cell number of primary nematogens is 36, that of primary rhombogens 31. This was based on study of insufficient material. Individuals of both phases exhibited variation in cell number over most of the range.

Dicyemennea granularis n. sp.

(Pl. 1, fig. 2; pl. 2, figs. 28-29)

Diagnosis.—Adults usually 1 to 4 mm. long, broad and robust-appearing, often exceeding 100 microns in width; total number of somatic cells 29 to 41, usually 30 to 35; trunk dark-appearing in life owing to heavy accumulation of proteinaceous eosinophilic granules in the somatic cells except those of the calotte, uropolar cells, and any verruciform cells present; calotte conical, orthotropical, generally narrower than the trunk, from which it projects as a clear protuberance; cephalic swelling composed of the calotte, the parapolar, and the first few pairs of diapolar cells; parapolar cells short, seldom exceeding the calotte in length; uropolar cells usually verruciform, 1 to 3 additional verruciform cells sometimes present; axial cell ends anteriorly between the bases of the metapolar cells.

Vermiform larvae with abortive second axial cell just anterior to the end of the axial cell, between the basal ends of the metapolar cells, may attain length of 0.1 mm. or more before leaving the axial cell of the parent nematogen.

Rhombogens tend to be larger and broader than nematogens, with large, very prolific infusorigens; axial cell with same number of paranuclei as the number of infusorigens present; somatic cells of trunk in this phase with from 1 to 4 accessory nuclei.

Infusoriform 30 to 36 microns long, tapering to a conical point posteriorly, each cell in the urn with 2 nuclei and 1 germ cell.

Host.—*Octopus bimaculatus* Verrill and *O. bimaculoides* Pickford and McConnaughey.

Distribution.—Southern California.

Type specimens.—Syntypes on slide L-8, 2. Author's collection. Paratypes, other slides of the L-8 series.

This species resembles *D. californica* in size and cell number. It is distinguished by the fact that some or most of the somatic cells of the trunk are heavily charged with characteristic granules. Also it is generally broader, more robust-appearing, and the anterior diapolar cells play a more prominent part in the cephalic swelling, the calotte and parapolar cells a less prominent role.

Dicyemennea abelis n. sp.

(Pl. 1, figs. 8-10; pl. 2, figs. 24-27)

Diagnosis.—Adults 0.5 to 2.5 mm. long, usually not over 1.5 mm.; total number of somatic cells 23 to 29, most often 24 to 27; body slender, of about the same width throughout, transparent in life except for the parapolar, uropolar, and verruciform cells; diapolar cells of rhombogens sometimes heavily charged with granules; cephalic swelling comprised of the calotte, parapolar, and sometimes the first pair of diapolar cells, may sometimes be lacking, especially in rhombogens, owing to elongation and narrowing of the metapolar and parapolar cells; somatic cells of trunk tend to be arranged in opposed pairs but not rigidly so; axial cell ends anteriorly between the basal ends of the metapolar cells.

Vermiform larvae short and stocky, with sharply pointed calotte; the abortive second axial cell just anterior to the axial cell proper between the bases of the metapolar cells; larvae generally attaining a length of only 50 or 60 microns before their escape from the parent nematogen.

Rhombogens with accessory nuclei in some of the somatic cells of the trunk, the axial cell containing the same number of paranuclei as the number of infusorigens present; metapolar and parapolar cells tend to be elongated and narrowed so that there is no swelling at the anterior end.

Infusoriform 40 to 45 microns long, rounded posteriorly, each cell within the urn containing two free nuclei and one germ cell.

Chromosome number.—Diploid number estimated to be about 18 to 20.

Host.—*Octopus bimaculatus* Verrill.

Distribution.—Coast of southern and Lower California.

Type specimens.—Syntypes on slide 106-2. Author's collection. Paratypes, other slides of the 106 series.

This species resembles *D. californica* but is smaller, with fewer somatic cells, and the vermiform larvae are much smaller and stockier when fully developed within the parent nematogens. The tendency for the metapolar and parapolar cells to elongate at the beginning of the rhombogen phase is more pronounced in this species. The infusorigens are smaller and the number of embryos contained in a parent nematogen or rhombogen is more limited.

Dicyemennea adscita n. sp.

(Pl. 1, fig. 6; pl. 3, fig. 37)

Diagnosis.—Adults mostly 1 to 3 mm. long; total number of somatic cells 23, sometimes more; body slender, of about the same breadth throughout or slightly constricted for a short distance behind the cephalic swelling, transparent in life except the parapolar, uropolar, and verruciform cells; commonly 2 verruciform cells in addition to the uropolars, all verruciform cells characterized by the presence of many small, irregularly shaped, deeply staining granules scattered in the cytoplasm between the vacuoles; cephalic swelling moderate, composed of the calotte and parapolar cells; calotte orthotropical, short, generally wider than long, the propolar cells about one-third to one-half the diameter of the metapolars; metapolars only slightly longer than wide; parapolar cells about equal to the calotte in length, rounded posteriorly, adjoined by 1 dorsal and 1 ventral trunk cell; somatic cells

of trunk, including the parapolars, arranged in 7 opposed pairs; axial cell ends anteriorly between the basal ends of the metapolar cells.

Rhombogens with only a few accessory somatic nuclei; the axial cell with the same number of paranuclei as the number of infusorigens present.

Infusoriform 33 to 36 microns long, bluntly pointed behind, each cell within the urn containing 2 free nuclei and 1 germ cell.

Chromosome number.—Diploid number estimated to be about 16.

Host.—*Octopus* sp.

Distribution.—Known only from an octopus dredged from a sand-and-mud bottom in about 35 fathoms of water off Point Loma, San Diego County, California. This octopus also contained *Dicyema balamuthi*. Five specimens of this species of *Octopus* were taken. They differed from *O. bimaculatus* in being of a lighter, more yellowish color, lacking the ocellations at the bases of the third arms, and in the hectocotylus. The specimens were submitted to Dr. S. S. Berry of Redlands, who reports that they belong to an undescribed species. One of the five contained *Dicyemenea adscita* and *Dicyema balamuthi*. The other four contained *Dicyema balamuthi* and *Conocyema adminicula*.

D. adscita resembles *D. abelis*, from which it differs in slightly larger average size, lower and more constant cell count, generally shorter and broader calotte, and more prominent verruciform cells with characteristic dark-staining inclusions. The uropolar cells are not as constantly verruciform.

Dicyemenea abasi n. sp.

(Pl. 1, fig. 14; pl. 3, figs. 38–39)

Diagnosis.—Adult nematogens 0.3 to 0.9 mm. long; total number of somatic cells variable, from 22 to 26, different larvae in the same nematogen sometimes varying over most of the range; body generally widest in the anterior third of the trunk, tapering slightly posteriorly, or of nearly equal breadth throughout; no verruciform cells or heavy accumulations of granules; calotte orthotropical, conical, generally narrower than the anterior part of the trunk; propolar cells about one-third to one-half as large as the metapolars; axial cell ends anteriorly between the basal ends of the metapolar cells; vermiform larvae with rudimentary second axial cell between the bases of the metapolar cells. Rhombogen unknown.

Host.—*Octopus* sp., probably *bimaculoides* Pickford and McConnaughey.

Distribution.—Balboa Bay, Orange County, California.

Type specimens.—Syntypes on slide 103-14. Author's collection. This slide also contains *Dicyemenea abbreviata* and *Dicyema acciaccatum*. They are all very small species, but are so distinctive that there is no likelihood of confusing them.

Dicyemenea brevicephala McConnaughey, 1941

(Pl. 1, fig. 7; pl. 3, figs. 33–36)

Diagnosis.—Small dicyemids, adults usually not exceeding 1 mm. in length although occasional individuals may reach as much as 2 mm.; total number of somatic cells 27, rarely 26 or 28; body broad, of about equal breadth throughout; calotte flattened, discoid, capping the broad truncated ends of the large parapolar cells, often plagiotropic; propolar cells much smaller than the metapolar cells which surround them, the whole forming a flattened or convex disk with the propolars in the center, seldom exceeding the trunk in diameter, cilia of calotte shorter and denser than on the trunk; axial cell broad, extending to the propolar cells, ending bluntly anteriorly; somatic cells of trunk sometimes charged with refractile granules; verruciform cells 0 to 3, not prominent.

Rhombogens slightly larger than nematogens, with accessory nuclei in some of the trunk cells; axial cell with same number of paranuclei as the number of infusorigens present.

Infusoriform generally 35 to 40 microns long, rounded posteriorly, each cell within the urn containing 2 nuclei and 1 germ cell, the latter often elongated and pycnotic in appearance in mature infusoriforms. The refringent bodies are very large, occupying about one-

half the body length. The granules elaborated by the capsule cells are large, attaining almost the size of the nuclei within the urn cells.

Chromosome number.—Diploid number estimated at about 18 or 20.

Host.—*Octopus apollyon* (Berry).

Distribution.—Marine View Rocks, San Mateo County; and Monterey Bay, California.

Dicyemenea abbreviata n. sp.

(Pl. 1, fig. 11; pl. 3, figs. 30–32)

Diagnosis.—Adults rarely exceeding 1 mm. in length; total number of somatic cells constant, 25; body short, stout, of about the same diameter throughout its length, truncated at the ends; calotte plus parapolar cells forming a cephalic swelling sometimes exceeding the width of the trunk and sharply set off from it by a strong constriction; metapolar and parapolar cells extend forward peripherally to or ahead of the small propolar cells, the whole complex forming a flattened or even concave disc with the 4 small propolar cells in the center, ringed by the 5 larger metapolar cells, these in turn by the crescentic parapolar cells; calotte proper set apart by markedly greater affinity for stains so that it forms a dark plaque contrasting with the rest of the body in stained preparations. Parapolar cells, though included in the cephalic swelling, stain more like other trunk cells; somatic cells of trunk, including the parapolars, arranged in 8 pairs, the caudal pair usually slightly inflated and verruciform.

Vermiform larvae with rudimentary second axial cell just anterior to the axial cell proper near the base of the propolar cells.

Rhombogens with indefinite number of accessory nuclei in some of the somatic cells of the trunk, the axial cell containing the same number of paranuclei as the number of infusorigens present, usually only 1 or 2.

Infusoriforms 37 to 41 microns in length, with 2 free nuclei and 1 germ cell in each of the cells within the urn; only a few infusoriforms present at any one time in a rhombogen, owing to the limited size of the vermiform individuals and the fact that the infusoriforms are not correspondingly reduced in size.

Chromosome number.—Diploid number estimated to be about 16 to 20.

Host.—*Octopus* sp., probably *bimaculoides* Pickford and McConnaughey.

Distribution.—Balboa Bay, Orange County, California.

Type specimens.—Syntypes on slide 103-14. Author's collection.

In size, cell number, and bodily characteristics this species is intermediate between *Dicyemenea brevicephala* and *Conocycema adminicula*.

Genus DICYEMA von K  lliker, 1849

Diagnosis.—Calotte 8-celled, 4 propolar and 4 metapolar cells, the 2 sets corresponding in position relative to the planes of symmetry of the calotte (except in *D. truncatum*), often plagiotropal in the adults; vermiform larvae without a small second axial cell.

Type species.—*Dicyema paradoxum* von K  lliker, 1849.

Synonym: *Dicyema clausianum* van Beneden, 1882.

KEY TO THE SPECIES OF DICYEMA*

1. Somatic cells usually 22 or more..... 2
- Somatic cells less than 22.....13
2. (1) Medium-sized or small species, rarely exceeding 4.5 mm..... 3
- Large species, commonly exceeding 5 mm.....12

* Subsequent to the preparation of this manuscript and its submission for publication, an additional species of *Dicyema* was found on slides from an immature octopus from Lower California. This species was named *D. sullivanii* and described by the writer in a separate article—McConnaughey (1949). At that time the distinction between *Octopus bimaculatus* and *O. bimaculoides* had not been made. It is reasonably certain that the host is *O. bimaculoides*, not *O. bimaculatus* as given. See Pickford and McConnaughey (1949).

3. (2) Axial cell extends forward to the propolar cells. 5
 Axial cell ends anteriorly at the base of or between the bases of the metapolar cells,
 not extending forward to the propolars. 4
4. (3) Calotte acutely pointed, propolar cells much smaller than the metapolars; nuclei
 with large irregular masses of chromatin. *acheroni* n. sp.
 Calotte not sharply pointed; propolar cells about half as large as the metapolars;
 nuclei with rounded nucleolus and only small peripheral granules of chromatin
 *acciaccatum* n. sp.
5. (3) Width of calotte approximately the same as or less than that of the anterior end of
 the trunk 6
 Width of calotte exceeds that of the anterior end of the trunk, from which it is
 well marked off 12
6. (5) Somatic cells characteristically 26, vary from 25 to 27.
 A robust, medium-sized species up to 2.5 mm. long, commonly with from 1 to 6
 verruciform cells; calotte hemispherical, orthotropical, not usually exceeding trunk
 in width. Hosts: *Octopus de filippi* Vérany and *Sepiolo steenstrupiana* Lévy.
 Distribution: Mediterranean. *microcephalum* Whitman, 1883
 Somatic cells characteristically 22. 7
7. (6) Calotte globular or elongate, seldom discoid, generally as long as or longer than
 broad 8
 Calotte usually flattened, somewhat discoid, wider than long; short stocky species 9
8. (7) Infusoriform with 2 nuclei and 1 germ cell in each cell within the urn; axial cell
 of vermiform individuals often swollen anteriorly within the calotte, especially
 in rhombogens. *apollyoni* Nouvel, 1947
 Infusoriform with 1 nucleus and 1 germ cell in each cell within the urn; axial cell
 of vermiform phases narrows rather than broadens within the calotte.
 Small, generally under 1 mm. Hosts: *Octopus vulgaris* and *Octopus fangsiao*.
 Distribution: near Misaki, Japan. *misakiense* Nouvel and Nakao, 1938
9. (7) Anterior ends of parapolar cells completely encircle axial cell. 10
 Anterior ends of parapolar cells do not completely encircle axial cell; first 2 dia-
 poles extend forward between parapolars to base of metapolar cells.
 Short stocky species, generally 0.5–1.5 mm. long; no distinct cephalic swelling;
 calotte of adults much wider than long, with very short metapolar and para-
 polar cells. Hosts: *Rondeletiola minor* Naef and *Sepietta oweniana* (d'Orb.).
 Distribution: Mediterranean. *rondeletiolae* Nouvel, 1947
10. (9) Metapolar cells regularly alternate with propolars in position relative to planes of
 symmetry of calotte; uropolar cells commonly verruciform.
 Short stocky species with small discoid calotte. Hosts: *Sepia officinalis* Linnaeus,
Sepia orbignyana Férussac, *Sepia esculenta* Hoyle and *Eosia macrosoma*
 (Della Chaije). Distribution: Mediterranean and Atlantic coasts of Europe;
 Japan *truncatum* Whitman, 1883
 Metapolar cells correspond with propolars in position relative to planes of sym-
 metry of calotte, or at least do not regularly and constantly alternate with them;
 uropolar cells not verruciform. 11
11. (10) Adults 0.5 to 1.0 mm. long; calotte discoid, small, telescoped; parapolar cells very
 short, not pointed behind; first diapolar cells make up most of the cephalic
 swelling; no true verruciform cells.
 Hosts: *Sepia elegans* d'Orb. and *Rondeletiola minor* Naef. Distribution: Medi-
 terranean. *schulzeianum* van Beneden, 1876
 Adults 1.5 to 3.6 mm. long; calotte nearly hemispherical in young, often flattened
 and much wider than long in adults; nuclei with large eosinophilic nucleoli.
 Hosts: *Sepioteuthis lessoniana* Férussac. Distribution: Japan
 *orientale* Nouvel and Nakao, 1938
12. (5) Calotte short, hemispherical, usually broader than long, forming well-marked
 cephalic swelling; propolar cells about same size as or even larger than meta-
 polars; commonly with 1 to 5 verruciform cells; somatic cells 25 to 28.
 Length: 2 to 3 mm. Hosts: *Octopus macropus* Risso and *O. vulgaris* Lamarck.
 Distribution: Mediterranean and Atlantic coasts of Europe, and Mauritania,
 French West Africa. *paradoxum* von Kolliker, 1849
 (Synonym: *clausianum* van Beneden, 1882)

- Calotte elongate, propolar cells usually smaller than metapolars; no verruciform cells; somatic cells 22.....*balamuthi* n. sp.
13. (2) Calotte usually flattened and oblique, with rounded or quadrilobate face exceeding diameter of trunk, free edges of metapolar cells extending out as platelike margin; somatic cells 28 to 33, usually 30 or 31.
 Large, sometimes up to 7 mm., relatively thin; 0 to 5 verruciform cells. Hosts: *Scpietia oweniana* (d'Orb.), *Scpietia obscura* Naef, *Sepiola steenstrupiana* Lévy, and *Sepia elegans* d'Orb. Distribution: Mediterranean
*macrocephalum* (van Beneden, 1878)
 Calotte variable but not flattened or platelike; somatic cells typically 24, varying from 22 to 26.
 Long slender species often 5 to 6 mm.; commonly with 1 or 2 large verruciform cells. Hosts: *Eledone moschata* (Lamarck) and *Sepiola rondeleti* Steenstrup. Distribution: Mediterranean.....*moschatum* Whitman, 1883
14. (1) Axial cell extends to base of propolar cells.....15
 Axial cell does not reach propolar cells.....16
15. (14) Somatic cells usually 18 or 19.
 Stocky; calotte usually not exceeding anterior end of trunk in width; metapolars widest posteriorly. Hosts: *Octopus vulgaris* Lamarck. Distribution: Mediterranean, Atlantic coast of Europe, North Sea.....*typus* van Beneden, 1876
 Somatic cells 16 or less.
 Small, thin; cephalic swelling (calotte plus parapolars) conspicuously wider than, and well marked off from, the thin trunk; metapolar cells often widest anteriorly. Hosts: *Octopus* sp. (either *vulgaris* or *rugosus*). Distribution: Mauritania, French West Africa.....*megaloccephalum* Nouvel, 1934
16. (14) Somatic cells 18 or 19; axial cell bluntly rounded anteriorly ending near the posterior ends of the metapolar cells; calotte conical, tapering to bluntly rounded point.
 Small, massive, less than 1 mm. long. Hosts: *Octopus vulgaris* Lamarck. Distribution: Japan.....*acuticephalum* Nouvel, 1947
 Somatic cells usually 16 or less; axial cell pointed anteriorly and insinuated farther forward between the metapolars but not reaching the propolars.
 Small, massive, seldom exceeding 0.5 mm.; cilia of calotte markedly shorter and more close set than on trunk. Host: *Octopus* sp. (*vulgaris* or *rugosus*). Distribution: Mauritania, French West Africa.....*monodi* Nouvel, 1934

Dicyema apollyoni Nouvel, 1947

(Pl. 4, fig. 48; pl. 5, figs. 51-55)

Diagnosis.—Adults 0.5 to 3.0 mm. long; total number of somatic cells 22; rhombogens usually broader and longer than nematogens; body broad, robust-appearing, transparent, not heavily granular, of nearly the same breadth throughout, or slightly wider at the anterior end; calotte orthotropical, broadly rounded or squarish, of about the same diameter as or somewhat exceeding the trunk; cephalic swelling composed of calotte, parapolar, and first pair of diapolar cells, often not well marked; propolar cells usually about equal in size to metapolars, sometimes exceeding them; parapolar cells subequal to calotte, shield-shaped, pointed behind; axial cell penetrates forward between bases of propolar cells, rounded or even inflated at anterior end, calotte fitting over it in manner of helmet; trunk cells disposed in opposed pairs but this arrangement often obscured in adults; rhombogens with variable number of accessory nuclei in some somatic cells of trunk; paranuclei of axial cell correspond in number with the number of infusorogens present, usually 1 or 2.

Infusoriforms 27 to 35 microns long, slightly tapered and conical behind, each refringent body about the same size as, or slightly larger than, the urn; each cell within the urn with 2 nuclei and 1 germ cell.

Chromosome number.—Diploid number probably 14.

Host.—*Octopus apollyon* (Berry).

Distribution.—Marine View Rocks, San Mateo County, California, and Monterey Bay, California.

Dicyema balamuthi n. sp.

(Pl. 4, figs. 46-47; pl. 5, figs. 56-64; pl. 6, figs. 71-79)

Diagnosis.—Adults generally 0.75 to 2.5 mm. long, largest measured specimen 4.0 mm.; total number of somatic cells 22; body thin, transparent, of about uniform diameter throughout except for the enlarged calotte, without verruciform cells; cephalic swelling composed almost entirely of the calotte; parapolar cells only slightly thickened at junction with calotte, often noticeably unequal; calotte elongate, well marked off from, and broader than, the trunk; propolar cells commonly about two-thirds to three-fourths as long as metapolars; somatic cells of trunk, including the parapolars, disposed in 7 opposed pairs; axial cell penetrates calotte to, or between bases of, the propolar cells, its anterior end inflated in mature individuals.

Hosts.—*Octopus* sp. First found in a small octopus by Dr. William Balamuth in the San Francisco Bay area, the octopus not preserved, probably a small *O. apollyon* (Berry). Subsequently found in five specimens of an unidentified species of octopus dredged from about 35 fathoms off Point Loma in San Diego County, California. These octopuses have already been discussed in connection with the description of *Dicyemennaea adscita*. A small red octopus dredged off Point Loma also contained numerous stem nematogens and young primary nematogens which have been tentatively assigned to this species.

Type specimens.—Syntypes on slide L-2, 6. Author's collection. Paratypes on other slides of the L-2 series. *Conocyema adminicula* is also present on these slides.

The axial nucleus in this species sometimes gives rise to additional free nuclei in the axial cell, through amitotic division, even in very young individuals, so that the number of free nuclei present in the axial cell of rhombogens may exceed the number expected from the number of infusorigens present, and they may also be encountered in the other phases before the formation of any infusorigens. The formation of nucleus-like bodies, other than paranuclei, from axoblasts during the period of transition of phase has also been noted.

The stem nematogen is typical of those of the genus *Dicyema*, having three axial cells in linear series, the anterior one lagging slightly behind the other two in its development. The calotte is somewhat flattened in the adult, the four metapolar cells alternating with the propolar cells. The total number of somatic cells in the clearest examples seen was 26. Old individuals may reach a length slightly exceeding 2 mm.

The vermiform larvae contained in the axial cells of mature stem nematogens are typical of those of the primary nematogens of this species.

Dicyema acciaccatum n. sp.

(Pl. 4, fig. 49; pl. 5, figs. 65-67)

Diagnosis.—Small dicyemids seldom reaching 1 mm. in length, the majority of mature individuals less than one-half mm. long; characteristic number of somatic cells 22 but occasional higher counts up to 28 observed; body stocky, of nearly the same breadth throughout, or sometimes broadest a short distance back of the calotte, tapering slightly towards both ends; axial cell ends anteriorly at the base of the metapolar cells; calotte elongate, not exceeding the trunk in diameter; propolar cells noticeably smaller than the metapolars; parapolar cells about equal to the calotte in length, adjoined posteriorly by 1 dorsal and 1 ventral trunk cell; cells of the trunk disposed as a rule in opposed pairs; no verruciform cells; the tendency to produce accessory nuclei at the beginning of the rhombogen phase is pronounced, starting early and producing more accessory nuclei than

in most species; the paranuclei and also the axial nucleus may divide amitotically so that the number of free nuclei in the axial cell often considerably exceeds that expected from the number of infusorigens present; usually only 1 or 2 infusorigens; infusoriform 25 to 37 microns in length, rounded posteriorly, each cell within the urn containing 2 nuclei and 1 germ cell, the germ cell lightly staining except for 2 or 3 large deeply staining blocks of chromatin.

Host.—*Octopus* sp., probably *bimaculoides* Pickford and McConnaughey.

Distribution.—Balboa Bay, Orange County, California.

Type specimens.—Syntypes on slide 103-14. Author's collection.

This species differs from the majority of its congeners in that the axial cell terminates near the base of the metapolar cells rather than extending forward to the propolars. It differs from *D. monodi* and *D. acheroni* in size, proportions, cell number, cytological aspect of the nuclei, and other details; from *D. megalocephalum* in cell number and in the shape of the body and calotte.

Dicyema acheroni n. sp.

(Pl. 4, fig. 50; pl. 5, figs. 68-70)

Diagnosis.—Small to medium-sized dicyemids, adults mostly 0.5 to 1.5 mm. long; body slender, of approximately the same breadth throughout or slightly broader near the anterior end of the trunk; calotte orthotropical pointed, slightly longer than broad, not exceeding the anterior end of the trunk in breadth; propolar cells markedly smaller than the metapolars; axial cell ends bluntly at the posterior end of the metapolar cells, not extending forward to the propolars; trunk cells not conspicuously charged with granules and none of those examined showed verruciform cells; all nuclei with coarse irregular pieces and strands of chromatin peripherally, very different from the usual rounded nucleolus and small chromatin granules of most species; somatic cells vary in number from about 23 to 28.

The infusoriforms seem to have unusually elongated bodies but they were partly obscured by precipitation of stain around the refringent bodies and no precise details could be made out.

Host.—*Octopus* sp., probably *bimaculoides* Pickford and McConnaughey.

Distribution.—Balboa Bay, Orange County, California.

Type specimens.—Syntypes, slide 113-9. Author's collection.

The ending of the axial cell behind the metapolar cells separates this species from all others except *D. monodi*, *D. acciaccatum*, and *D. acuticephalum*, from which it differs in cell number, aspect of the nuclei, shape of the calotte, and proportions.

Genus CONOCYEMA van Beneden, 1882

Synonym: *Microcyema* van Beneden 1882

Diagnosis.—Dicyemids in which the axial cell of the adult is branched or irregular in shape. The somatic cells of some species may lose their cilia and fuse into a sort of syncytium in which individual cell boundaries cannot be distinguished. The known species are all small, with a constant number and arrangement of cells in the larvae.

The larvae are short, robust, usually with a strong constriction dividing the body into an anterior region composed of the calotte plus the parapolar cells, and a posterior region composed of the axial cell and the cells of the trunk enveloping it. The cells of the calotte stain more intensely than the others and may bear a prominent tuft of cilia directed forward. The remaining somatic cells bear cilia directed laterally or posteriorly. The total number of somatic cells in the species now known is smaller than in most ordinary dicyemids.

The infusorigens and infusoriforms are typical of those of dicyemids generally, showing no special distinguishing characteristics.

As here treated, this genus includes all the so-called heterocyemids. Since only three species are known, and these incompletely, it has seemed best to group them into one genus in view of the larval and adult characteristics which they have in common, rather than to follow previous authors in recognizing a separate genus for each species. If more species are discovered in the future and their relationships more clearly worked out, a sound basis for generic divisions may become apparent. In the present paper the generic name of the first described species is recognized as the proper generic name for the group.

Type species.—*Conocyema polymorpha* van Beneden, 1882.

KEY TO THE SPECIES OF CONOCYEMA

1. Somatic cells 10, only 2 of them posterior to the postcephalic constriction in the larvae. Adult very irregular and variable in shape, without cilia and with somatic cells fused into a syncytium in which cell boundaries are difficult or impossible to make out.
 Host: *Sepia officinalis* L. Distribution: Mediterranean and Atlantic coasts of Europe.....*vespa* van Beneden, 1882
 Somatic cells more than 10 in number, more than 2 of them posterior to the post-
 cephalic constriction in the larva.....2
2. Somatic cells 12 (possibly 16), 4 large inflated anterior cells followed by 8 trunk cells. Adults irregular in shape, without cilia.
 Host: *Octopus vulgaris* Lamarck. Distribution: Mediterranean
*polymorpha* van Beneden, 1882
 Somatic cells 17, cephalic swelling of 11 cells, followed by filiform trunk with 6
 somatic cells.....*adminicula* n. sp.

Conocyema adminicula n. sp.

(Pl. 1, fig. 15; pl. 3, figs. 40-45)

Diagnosis.—Small dicyemids mostly not exceeding 1 mm. in length; total number of somatic cells 17; adult nematogens of striking appearance, the trunk slender and filiform as in typical dicyemids, the anterior end, comprising the calotte plus the parapolar cells, greatly inflated, giving the organism as a whole the appearance of a flower on a stalk; metapolar cells especially inflated, each invaded by a branch from the anterior end of the axial cell; in old nematogens the portion of the axial cell contained in the swollen calotte may exceed that in the trunk and contain the axial nucleus together with most or all of the germ cells and developing embryos; metapolar and parapolar cells vacuolated and sometimes with large round eosinophilic bodies, presumably material derived from host tissue cells in the course of digestion, attached to the surface and inside the cells in vacuoles.

Vermiform larvae short, robust-appearing, without any abortive second axial cell; a noticeable constriction at the base of the parapolar cells; calotte staining more intensely than the remainder of the body, forming a dark plaque at the anterior end; body of the larva entirely ciliated, those on the calotte prominent and directed forward; cephalic swelling comprised of 11 cells, 4 propolars, 5 metapolars, and 2 parapolars; the remainder of the body forming the trunk composed of the axial cell and 6 enveloping somatic cells; the number and arrangement of cells very constant.

Hosts.—At least two, possibly three, species of unidentified *Octopus* dredged off Point Loma, San Diego County, California, and from near the Coronado Islands. The octopuses have been submitted to Dr. S. S. Berry of Redlands.

Type specimens.—Syntypes, slides of the L-11 series. Author's collection.

This species is intermediate in structure between the typical dicyemids of the genus *Dicyemenea* and the heterocyemids previously known, exhibiting both the filiform trunk characteristic of the former, together with the branching irregular shape of the axial cell, anteriorly at least, and the larval char-

acteristics of the latter. It appears to be most closely related to *Dicyemenea abbreviata* which may illustrate the first definite step toward the heterocyemid condition.

THE STATUS OF THE FAMILY CONOCYEMIDAE*

Gersch (1938) maintained that the forms described by van Beneden (1882), and subsequently observed by Lameere (1916, 1918, 1919) and Nouvel (1936, 1937) as "heterocyemids," actually represent stages in the degeneration of old individuals of ordinary dicyemids or misinterpretation of damaged or poorly fixed specimens. He claimed to have found all intermediate conditions between the true dicyemids and the heterocyemids among degenerating individuals of *Dicyema typus* and *Dicyemenea eledones*.

Hartmann (1939) briefly reviewed this question and pointed out that, while Gersch's illustrations do correctly represent degenerating individuals of ordinary dicyemids, they do not at all represent the heterocyemids. The distinctive characters of the young larvae, for one thing, were entirely ignored by Gersch, as well as the question of relative sizes. From his study of the literature and the fact that he too on one occasion had found heterocyemids, Hartmann concluded that Gersch was entirely mistaken on this question.

Schartau (1940) published a study of heterocyemids found by him at Naples and concluded that the heterocyemids are good species and the family a valid one.

Nouvel (1947) has summarized the findings of all previous authors with regard to the heterocyemids and treats them as a valid family.

My own study of the question, together with the fact that I have found heterocyemids showing clearly all ages from young vermiform embryos to adult nematogens and young rhombogens, enables me to confirm the views of Hartmann, Schartau, Lameere, and Nouvel on this question. There can be no doubt that the organisms described as heterocyemids are species in their own right and not degenerating individuals of other dicyemids.

It is uncertain whether they constitute a natural group, or simply represent an extreme condition, characterized by reduction of the trunk and expansion of the axial cell into the greatly inflated cephalic swelling, which condition may have arisen independently from the two common genera.

Conocyema adminicula seems clearly related to the genus *Dicyemenea*. A series of species can be selected showing transitional characteristics in small steps from typical *Dicyemenea* such as *D. californica* to the heterocyemid *C. adminicula*. All are alike in having a calotte of 4 propolar cells and 5 metapolar cells and an elongated trunk. The series could be selected as follows:

Dicyemenea californica: Medium to large size; large, variable number of somatic cells (30 to 38); axial cell terminating anteriorly at the base of the metapolar cells; vermiform

* The name Heterocyemidae was given to these organisms by van Beneden (1882), and has been used by most subsequent investigators. Stunkard (1937) pointed out that this name was not a valid family name since there is no genus *Heterocyema*. He proposed the name Conocyemidae in its place.

Since these species have been termed heterocyemids by almost all writers on the subject and this has become the familiar name for the group, its use is continued here for convenience.

larvae with an abortive second axial cell anterior to the definitive axial cell near the back of the metapolar cells; calotte not markedly different in staining characteristics from the trunk.

Dicyemennea abels: Similar to the foregoing but smaller and with a lower cell count.

Dicyemennea brevicephala: Smaller size; greater cell constancy, cell number nearly always 27; axial cell extends forward to the base of the propolar cells; abortive second axial cell of larvae well forward next to the propolar cells; calotte of adult flattened, disklike.

Dicyemennea abbreviata: Very small size; cell number rigidly constant, 25; calotte flat or even concave, deeply staining as compared to the trunk, its cilia directed forward; a prominent groove separating the cephalic swelling (calotte plus parapolar cells) from the trunk; abortive second axial cell of vermiform larvae well forward against the propolar cells.

Conocyema adminicula: Very small size; cell number rigidly constant, 17; no abortive second axial cell in the vermiform larva; separation of cephalic swelling from the rest of the trunk even more marked; axial cell branching into the much swollen metapolar cells in the adult, becoming very irregular in older individuals; filiform trunk retained.

Conocyema vespa might be thought of as continuing this series further, with still greater reduction of the trunk to two somatic cells and of the calotte to six. It is not clear to what other dicyemids it is most closely related.

Conocyema polymorpha is not described with enough definiteness to place it in this series with certainty. If the four large granular lobes at the anterior end represent metapolar cells rather than propolars, it might be considered to stand in much the same relation to *Dicyema* as *Conocyema adminicula* does to *Dicyemennea*.

Further study of *Conocyema polymorpha* and the description of additional species showing the heterocyemid condition are necessary before it can be decided whether the heterocyemids constitute a natural group or whether they arose in a polyphyletic manner as extreme forms from both the other genera.

The distinctive larval and adult characteristics of the heterocyemids are such as to make it convenient to group them together as a unit at present. Until the group is better known, there seems no good reason for recognizing separate genera for the three species. The writer agrees with Lameere that they are not sufficiently different from other dicyemids to warrant placing them in a separate family.

Genus DICYEMODECA Wheeler, 1897
(Pl. 7, figs. 80-89)

Wheeler (1897) named this genus for dicyemids found by him in the region of San Diego Bay, California, which differed from other known dicyemids in the possession of 10 cells in the calotte, the 4 propolar cells being followed by 6 instead of the usual 4 or 5 metapolar cells. He recognized one species for which he proposed the name *Dicyemodeca sceptrum*. Other students of the group have never reported such forms.

While restudying the dicyemids from the San Diego Bay region, the writer has confirmed Wheeler's observation that forms with 10 cells in their calotte do occur. However, their occurrence, so far as my observations go, is very rare and irregular—single individuals or only a few being found on slides con-

taining many ordinary dicyemids with which they seem in other respects conspecific. Thus "deca" individuals have been found on slides of *Dicyemennea californica*, *Dicyemennea granularis*, *Dicyemennea abelis*, and a single such specimen of *Conocyema adminicula*. All the abnormal specimens appeared otherwise conspecific with the predominant species on the slides. Furthermore, one large nematogen showing this condition contained several well-developed vermiform larvae all of which were normal and typical of *Dicyemennea californica*.

Twice, individuals of *D. californica* with 5 propolar cells were found, which thus arrived at a "deca" condition by a different means, and one of *D. granularis* in which both the propolar and metapolar groups of cells were increased by one, giving the calotte 11 cells.

It appears then that the genus *Dicyemodeca* must be regarded as founded upon aberrant individuals and cannot be recognized as a valid taxonomic category. Should this malformity prove hereditary and become established so that distinct and separate populations of *Dicyemodeca* occur, they might perhaps deserve recognition with as much justification as do the genera now recognized.

In the spontaneous appearance of this character in four different species there is seen the possibility of the polyphyletic origin of one or more of the genera now recognized.

NOTE ON THE OCTOPUS BIMACULATUS COMPLEX OF SOUTHERN CALIFORNIA

The cephalopods along the coast of southern California, which have been known as *Octopus bimaculatus* Verrill, actually represent two species differing most conspicuously in the size of the eggs and of the adults. In former publications on West Coast cephalopods, and on their parasites, Verrill's name was used for both forms. The smaller, large-egged octopus has been named *Octopus bimaculoides* Pickford and McConnaughey, 1949. Verrill's name is the valid one for the larger, small-egged form.

Unfortunately the octopuses from Balboa Bay, California, from which some of my earlier collections of mesozoa were made, were not preserved, so it is impossible to state with certainty to which species they belonged. Also no sure way has been found to determine to which species very young octopuses belong.

Some of the early Balboa Bay collections contained species of mesozoa not subsequently encountered (*Dicyemennea abasi*, *Dicyemennea abbreviata*, *Dicyema acciaccatum* and *Dicyema acheroni*). Since these octopuses were taken in a shallow mud flat area at Balboa Bay it seems probable that they were *O. bimaculoides*.

Wheeler (1897, 1899) studied the dicyemids of "*Octopus punctatus*" from the San Diego Bay region, California, and reported the presence of three species, which he named *Dicyema coluber*, *Dicyemennea whitmanii*, and *Dicyemodeca sceptrum*. It appears probable that the octopuses studied by

Wheeler comprised both species that have been confused under the name *O. bimaculatus* Verrill, and possibly a third species. He published no illustrations or descriptions of the dicyemids he named that are sufficient to permit definite identification. The genus *Dicyemodeca* appears to have been based on aberrant individuals of certain species of *Dicyemennae*. The specific names proposed by Wheeler are considered to be *nomina nuda*.

SUMMARY

Cephalopods of the Pacific coast in a section from San Francisco Bay to Rosarito Beach, Lower California, and including the Coronado Islands, have been examined for dicyemids. A small series of octopuses from the Hawaiian and Marshall islands were also examined, but were found not to be infected.

Twelve species of dicyemids, all distinct from those reported from Europe and Japan, were found. Descriptions and illustrations of these are presented. Nine of them are new species.

The existence of the heterocyemid group, denied by Gersch, is confirmed. The genus *Conocyema* is revised to include all of the three species now known.

The existence of the stem nematogen, denied by Gersch, is confirmed. The stem nematogen of *Dicyema balamuthi* is described.

Evidence is presented to show that the genus *Dicyemodeca* was founded on aberrant individuals and is not a valid taxonomic category.

There are two species of *Octopus*, common along the coast of southern California, which have been confused under the name *Octopus bimaculatus* Verrill.

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PLATES

PLATE 1

The known species of genus *Dicyemennea* and *Conocyema* all drawn to the same scale to show general characteristics of size and proportions. All figures except 3, 4, 5, 16, and 17 made with the aid of a camera lucida from preparations in the author's collection.

Fig 1 *Dicyemennea californica*

Fig 2 *Dicyemennea granularis*

Fig 3 *Dicyemennea eledones* (based on description by Whitman)

Fig 4 *Dicyemennea lameerei* (based on description by Nouvel)

Fig 5 *Dicyemennea gracile* (based on description by Whitman)

Fig 6 *Dicyemennea adscita*

Fig 7 *Dicyemennea brevicephala*, five normal and one giant individual

Figs 8 to 10 Groups of *Dicyemennea abels* taken from different individual octopuses, showing variation in size

Fig 11 *Dicyemennea abbreviata*

Fig 12 *Dicyemennea californica*, abnormal individual with six metapolar cells (see pl 7 for details)

Fig 13 *Dicyemennea californica*, another abnormal individual with six metapolar cells

Fig 14 *Dicyemennea abasi*

Fig 15 *Conocyema adumnicula*

Fig 16 *Conocyema polymorpha* (from description by van Beneden)

Fig 17 *Conocyema vespa* (from description by Lameere)

Fig 18 Small unnamed species of *Dicyemennea* for which there has not yet been sufficient material on hand from which to draw up a satisfactory description. From *Octopus bimaculatus* Verrill, Balboa Bay, California.

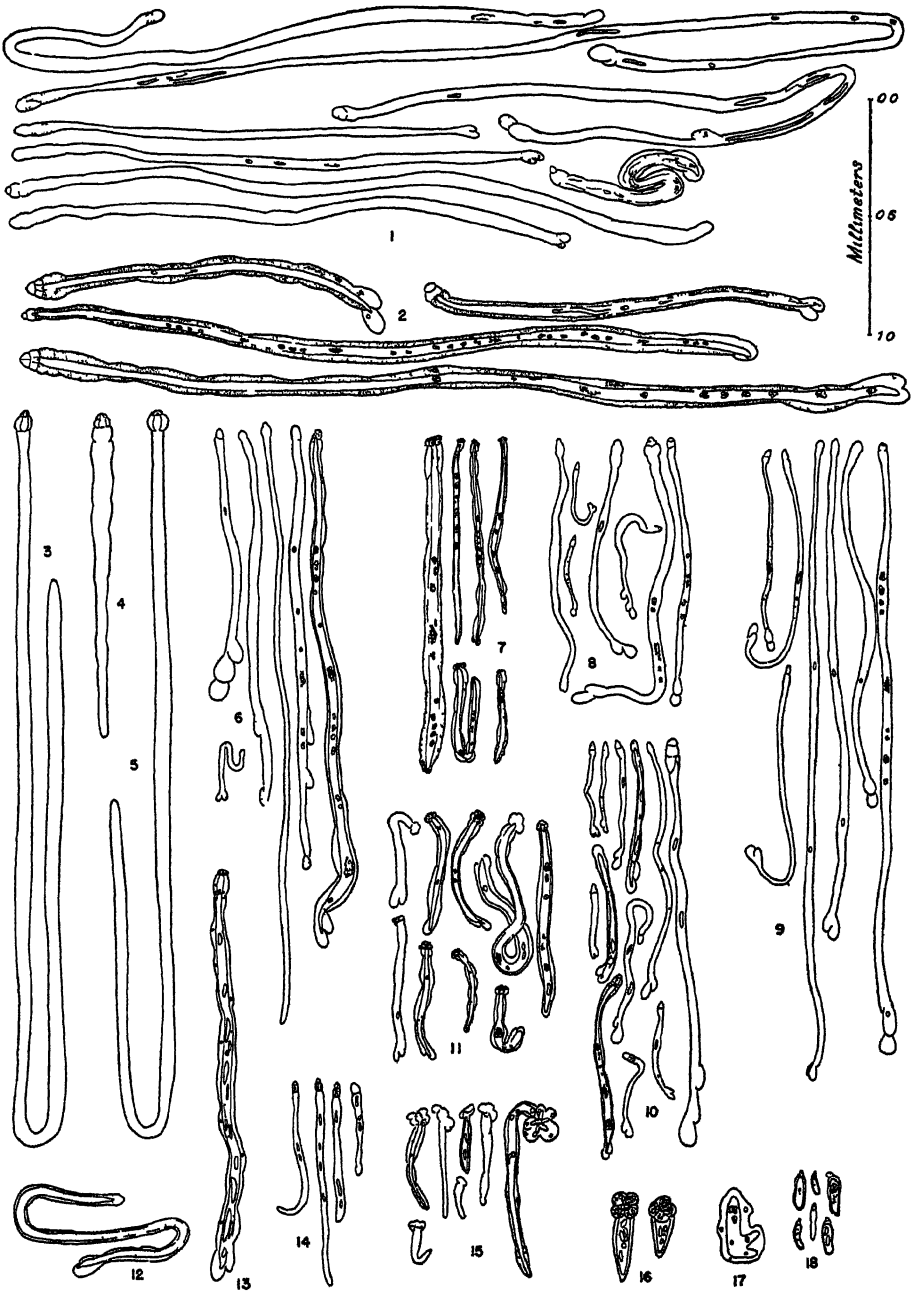


PLATE 2

Fig. 19. *Dicyemennea californica*, young nematogen (scale A).

Fig. 20. *Dicyemennea californica*, anterior end of an individual transitional between the nematogen and rhombogen phases, some fusiform axoblasts in the axial cell (scale A).

Fig. 21. *Dicyemennea californica*, anterior end of a large nematogen (scale A).

Fig. 22. *Dicyemennea californica*, vermiform larva inclosed in the axial cell of a nematogen (scale C).

Fig. 23. *Dicyemennea abelis*, vermiform larva inclosed in the axial cell of a nematogen (scale C).

Fig. 24. *Dicyemennea abelis*, young primary rhombogen showing binucleate axoblasts at either end of the row of axoblasts (scale B).

Fig. 25. *Dicyemennea abelis*, anterior end of a nematogen (scale A).

Fig. 26. *Dicyemennea abelis*, anterior end of a young rhombogen showing elongation of the metapolar and parapolar cells (scale A).

Fig. 27. *Dicyemennea abelis*, broad form of young vermiform individual shortly after escape from the axial cell of the parent nematogen (scale C).

Fig. 28. *Dicyemennea granularis*, anterior end of a rhombogen (scale A).

Fig. 29. *Dicyemennea granularis*, anterior end of a rhombogen (scale A).

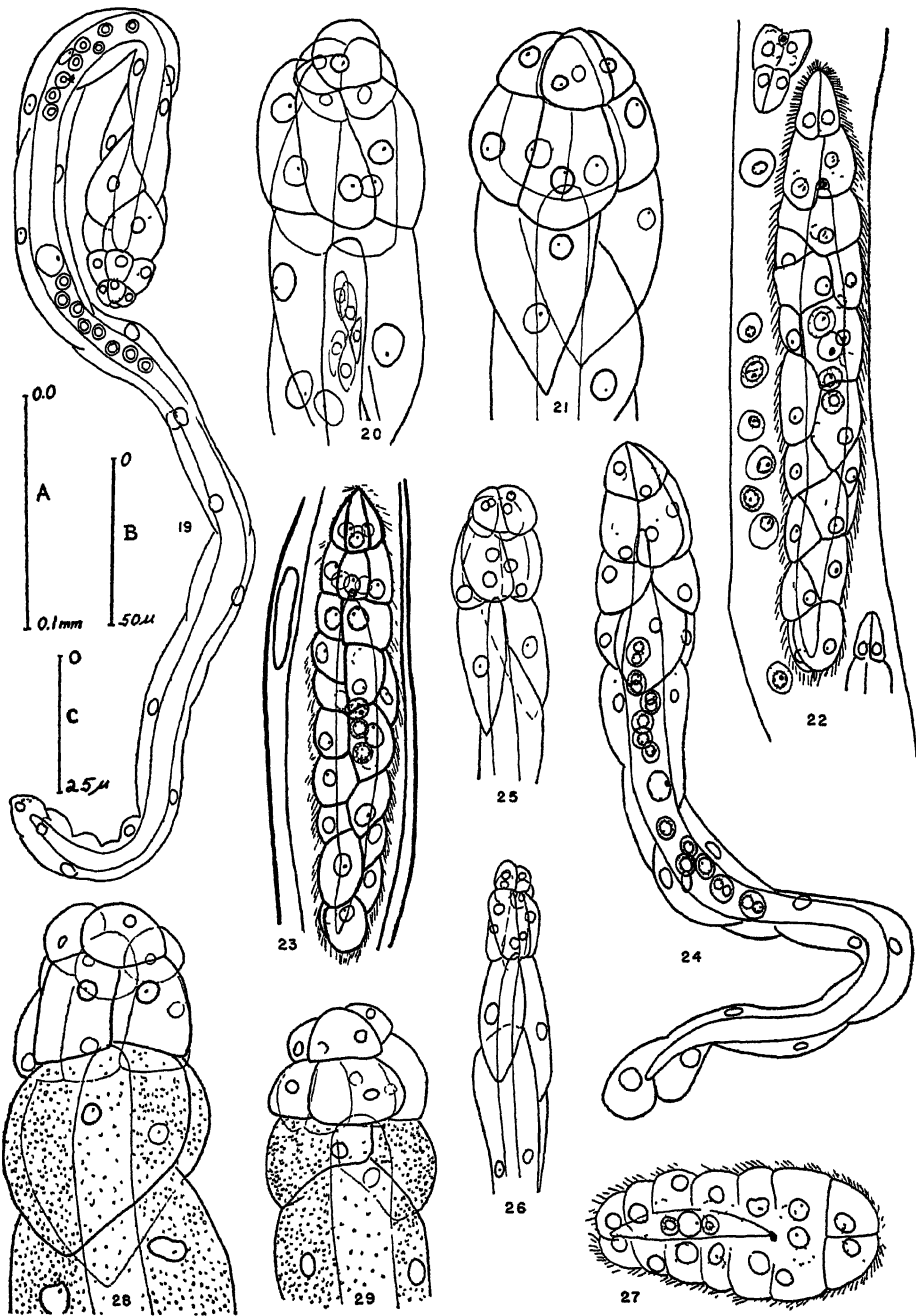


PLATE 3

Fig 30 *Dicyemennea abbreviata* nematogen (scale A)

Fig 31 *Dicyemennea abbreviata*, calotte and parapolar cells in polar view showing the disposition of the cells (scale B)

Fig 32 *Dicyemennea abbreviata*, optical frontal section of a young vermiform larva showing the small abortive second axial cell anterior to the axial cell proper, and the characteristic position of the axial cell nucleus in front of both of the first two axoblasts (scale D)

Fig 33 *Dicyemennea brevicephala* anterior end of a rhombogen (scale B)

Fig 34 *Dicyemennea brevicephala*, anterior end of a young rhombogen with the calotte oblique showing the disposition of the cells (scale B)

Fig 35 *Dicyemennea brevicephala*, anterior end of a young rhombogen (scale B)

Fig 36 *Dicyemennea brevicephala*, vermiform larva within the axial cell of a nematogen, the pycnotic remnant of the abortive second axial cell in late stage of degradation in one of the propolar cells (scale D)

Fig 37 *Dicyemennea adscita*, anterior end of a rhombogen (scale A)

Fig 38 *Dicyemennea abasi*, anterior end of a nematogen (scale B)

Fig 39 *Dicyemennea abasi*, anterior end of another nematogen (scale B)

Fig 40 *Conocyema adminicula* anterior end of a young nematogen, showing the branching of the axial cell into the metapolar cells (scale B)

Fig 41 *Conocyema adminicula*, anterior end of a young nematogen (scale D)

Fig 42 *Conocyema adminicula*, very young individual shortly after its escape from the parent nematogen (scale D)

Fig 43 *Conocyema adminicula*, young individual with the axial cell just beginning to branch into the metapolar cells, polar view of the cephalic swelling to show the disposition of the cells (scale D)

Fig 44 Optical section of the calotte of the same individual as in fig 43, showing the branching of the axial cell into the metapolar cells (scale D)

Fig 45 Optical section at the level of the parapolar cells to show the narrowing of the axial cell as it passes from the inflated calotte to the trunk

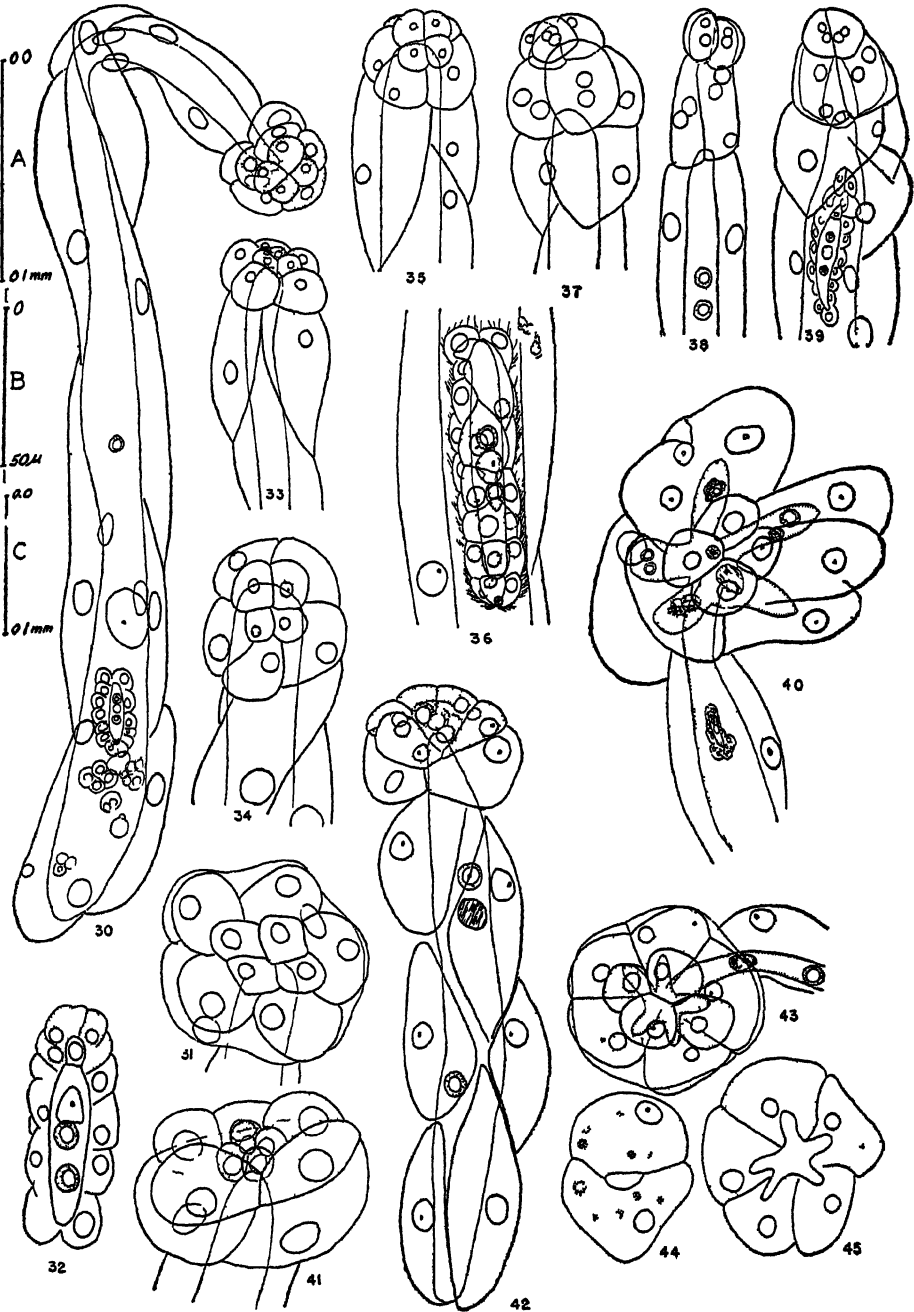


PLATE 4

The species of *Dicyema* from California, all drawn to the same scale to show general characteristics of size and form.

Fig. 46. *Dicyema balamuthi*, from off Point Loma, San Diego County, California.

Fig. 47. *Dicyema balamuthi*, from small octopus in the San Francisco Bay area.

Fig. 48. *Dicyema apollyoni*.

Fig. 49. *Dicyema acciaccatum*.

Fig. 50. *Dicyema acheroni*.

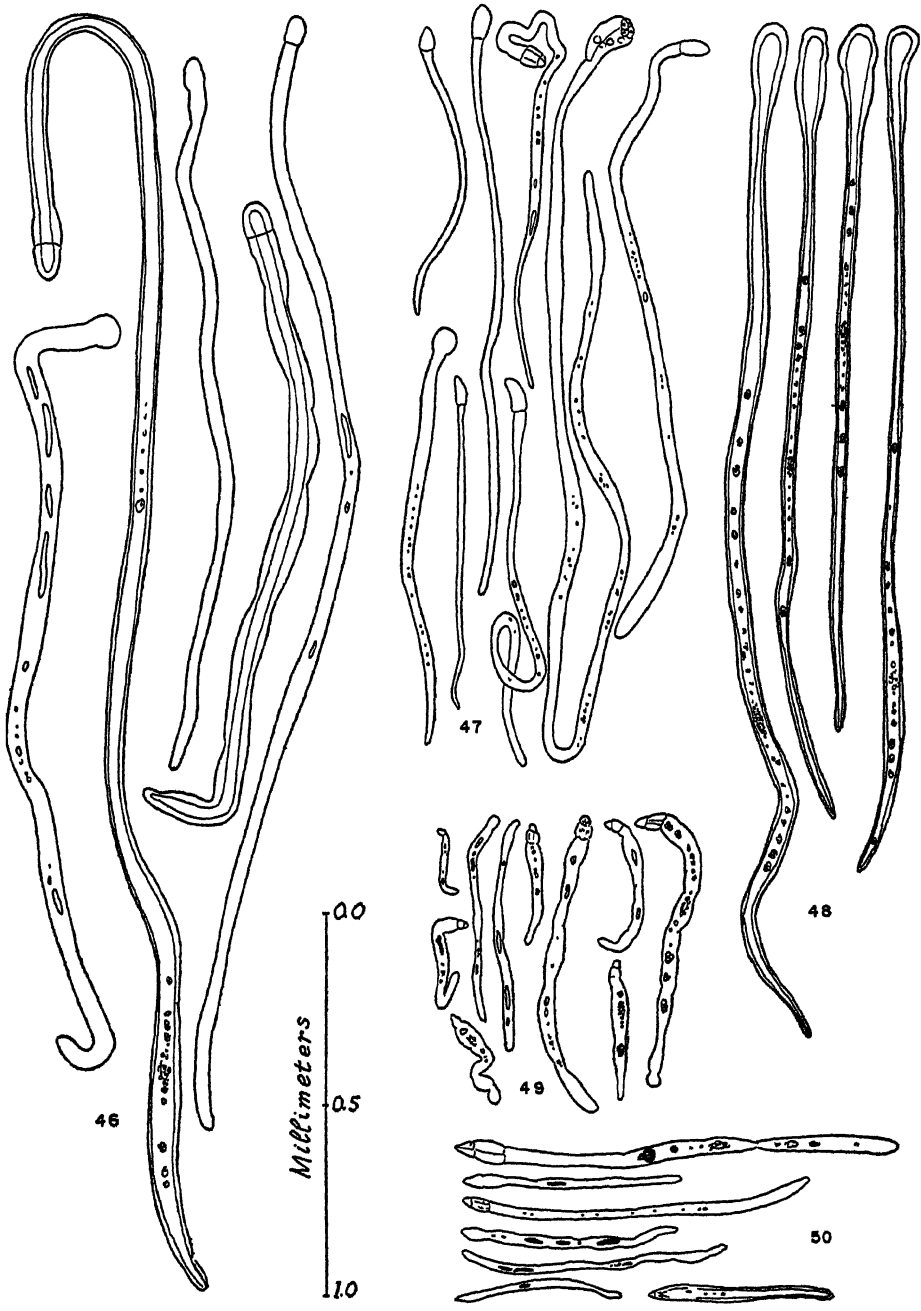


PLATE 5

Figs. 51-55 *Dicyema apollyoni*

Fig. 51. Anterior end of a mature rhombogen (scale B).

Fig. 52. Anterior end of another rhombogen. Amitotic division of nuclei of some of the trunk cells indicated (scale B).

Fig. 53. Anterior end of a rhombogen (scale B).

Fig. 54. Portion of a transitional individual showing some enlarged fusi-form germ cells (scale B).

Fig. 55. Vermiform larva (scale C).

Figs. 56-64 *Dicyema balamuthi*

Fig. 56. Anterior end of a transitional individual from off Point Loma (scale A).

Fig. 57. Anterior end of transitional individual from off Point Loma. Note inequality of parapolar cells common in this species (scale A).

Fig. 58. Elongated calotte, individual from San Francisco Bay area (scale A).

Fig. 59. Elongated calotte, individual from off Point Loma (scale A).

Fig. 60. Anterior end of nematogen from San Francisco Bay area (scale A).

Fig. 61. Anterior end of another nematogen from San Francisco Bay area with slightly elongated calotte (scale A).

Figs. 62-64. Vermiform larvae (scale C).

Figs. 65-67 *Dicyema acciaccatum*

Fig. 65. Anterior end of a rhombogen (scale B).

Fig. 66. Young primary rhombogen with some binucleate axoblasts (scale C).

Fig. 67. Young individual showing amitotic division of the axial nucleus into several parts (scale C).

Figs. 68-70 *Dicyema acheroni*

Fig. 68. Anterior end of a rhombogen (scale A).

Fig. 69. Anterior end of another rhombogen (scale A).

Fig. 70. Anterior end of a nematogen (scale B).

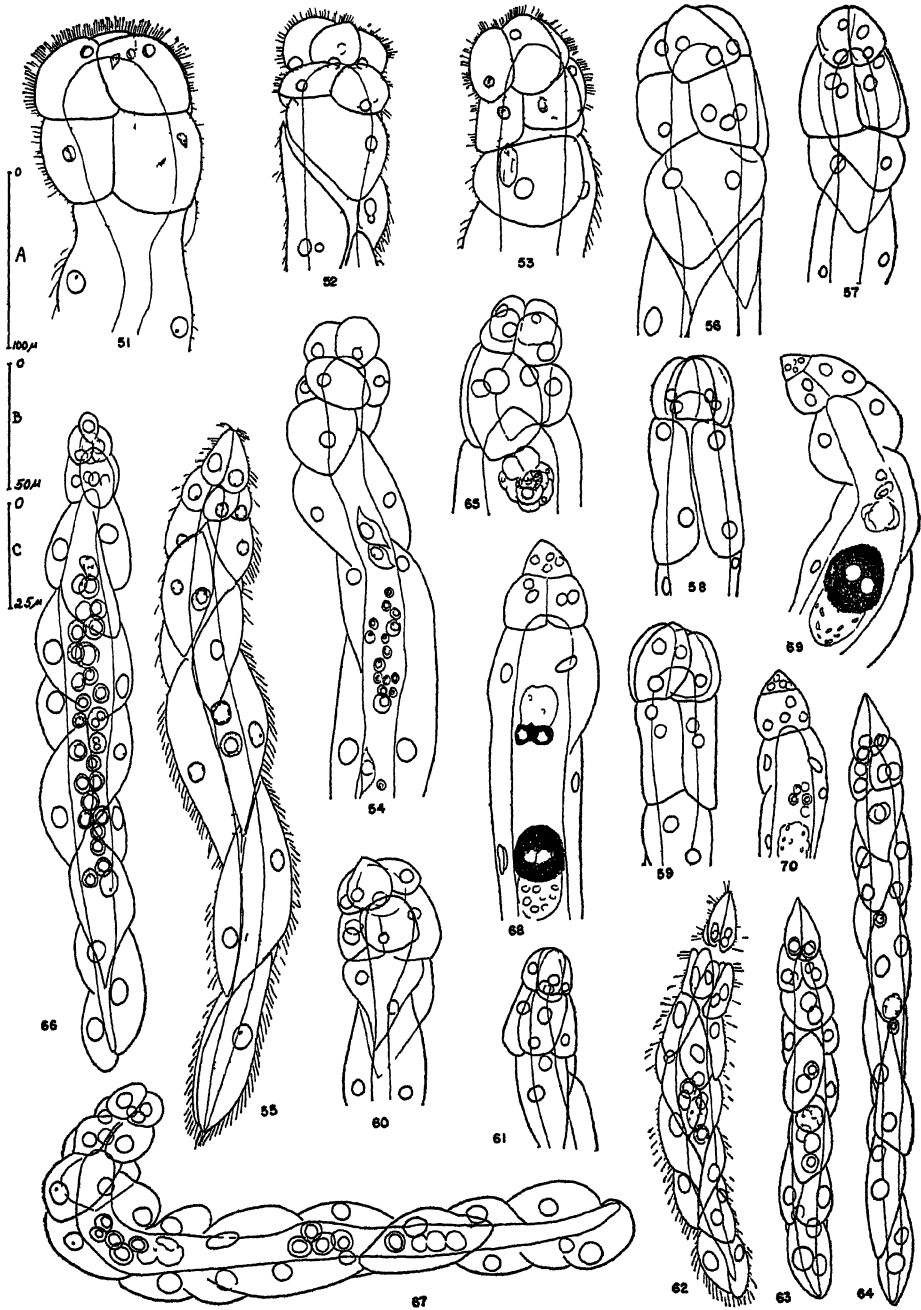


PLATE 6

The Stem Nematogen of *Dreyema balamuthi*

Fig. 71. Larval stem nematogen with only the axial nucleus and one axoblast in each axial cell. A number of pycnotic remnants of larval abortive cells are still present undergoing absorption in the anterior somatic cells.

Fig. 72. Young stem nematogen. Some multiplication of axoblasts has taken place and the abortive elements have been completely absorbed.

Fig. 73. Another young stem nematogen of about the same age.

Fig. 74. Stem nematogen in which the development of larval primary nematogens has started. The two posterior axial cells each contain two fully formed larval primary nematogens.

Fig. 75. Mature stem nematogen containing a large number of fully developed larval primary nematogens in each axial cell.

Fig. 76. Anterior end of the individual shown in fig. 75, to show the flattened calotte, the three parapolar cells and their relation to the axial cell.

Fig. 77. Very large mature stem nematogen with numerous larval primary nematogens in each axial cell.

Fig. 78. Younger stem nematogen in which all 26 somatic cells show clearly.

Fig. 79. Anterior end of the individual shown in fig. 78, showing the calotte and the three parapolar cells.

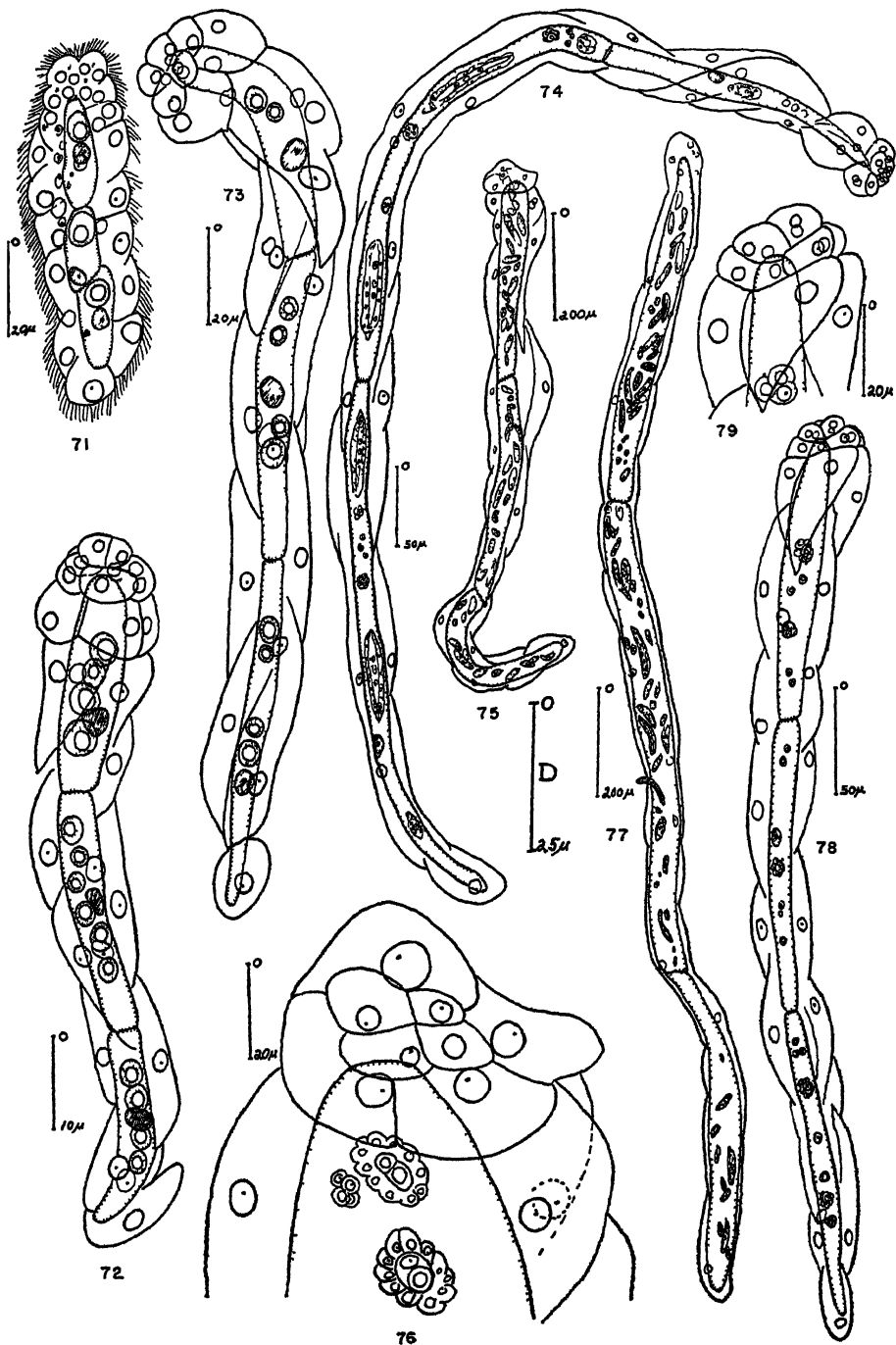


PLATE 7

The Basis for the Genus *Dicyemodēca* Wheeler Abnormalities of the calotte

Fig. 80. Anterior end of a nematogen of *Dicyemennea californica* with six metapolar cells (scale A).

Fig. 81. One of the many normal vermiform larvae contained in the axial cell of the nematogen figured in fig. 80, showing a normal calotte of four propolar and five metapolar cells (scale B).

Fig. 82. Anterior end of an individual of *Dicyemennea abelis* with six metapolar cells (scale A).

Fig. 83. A very young *Conocyema adminicula* with six metapolar cells (scale B).

Fig. 84. Anterior end of a rhombogen of *Dicyemennea granularis* with six metapolar cells (scale A).

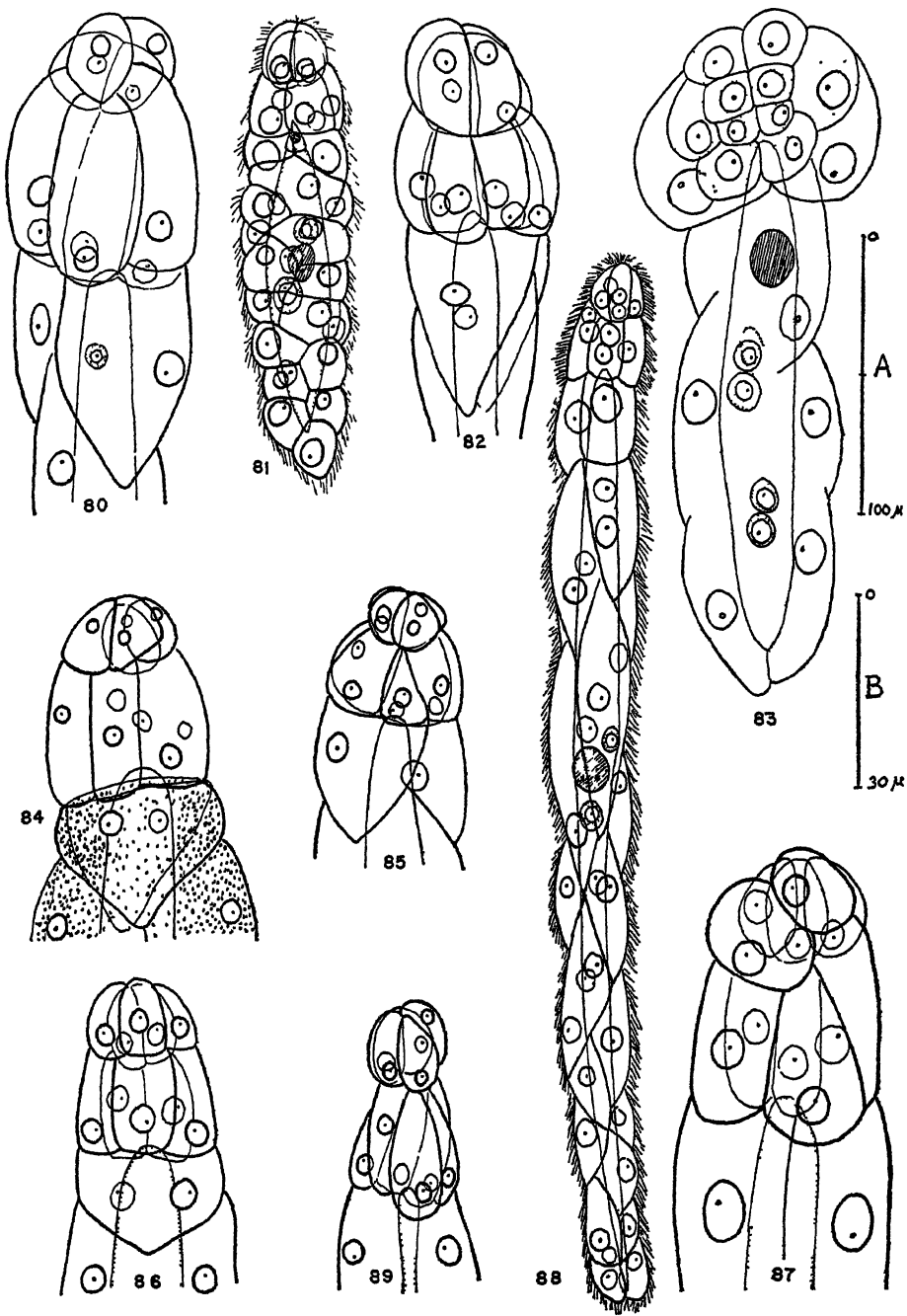
Fig. 85. Anterior end of a rhombogen of *Dicyemennea californica* with six metapolar cells (scale A).

Fig. 86. Anterior end of *Dicyemennea californica* with five propolar and five metapolar cells (scale A).

Fig. 87. Anterior end of *Dicyemennea californica* with five propolar cells, one of which is not well integrated with the calotte but shoved up between the propolar and metapolar cells (scale A).

Fig. 88. Larval *Dicyemennea californica* with five propolar cells. The parent nematogen and other larvae present were normal (scale B).

Fig. 89. Anterior end of a rhombogen of *Dicyemennea granularis* with five propolar and six metapolar cells, each set having been increased by one (scale A).



INTESTINAL FLAGELLATES
FROM A WALLAROO,
MACROPUS ROBUSTUS GOULD

BY
HAROLD KIRBY AND BRONISLAW HONIGBERG

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INTESTINAL FLAGELLATES FROM A WALLAROO, *MACROPUS ROBUSTUS* GOULD

BY

HAROLD KIRBY AND BRONISLAW HONIGBERG

INTRODUCTION

IN THE Zoölogical Gardens in San Francisco a number of wallaroos of the species *Macropus robustus* are maintained in pens. At intervals in the past four years examinations of the fecal pellets of the animals in one pen have been made to detect protozoan infection. Few flagellates have been found by direct observation of the fecal material, but most inoculations into a culture medium resulted in growth of flagellates. The culture medium consisted of a slant of coagulated whole egg covered with a fluid of 5 per cent horse serum in Ringer's solution. To this a loopful of rice starch was added. Transfer to fresh culture medium was made about every five days.

The predominant flagellate that developed in the cultures was a species of *Trichomonas*. This multiplied readily, reaching its maximum population growth after about sixty hours at 37° C., and could apparently be maintained indefinitely. A species of *Retortamonas* also developed, but often only in small numbers. It reached its maximum growth after about twenty-four hours. It has been kept through several transfers, but eventually disappeared in the presence of *Trichomonas*. On certain slides a few specimens of *Monocercomonas* sp. were found.

Living flagellates were studied by dark-field illumination. Flagellates in a drop of culture medium were placed on slides, covered, and the coverslip edges sealed with melted paraffin. On slides left at room temperature it was found that after forty-eight hours the state of reduced activity of *Trichomonas* and *Retortamonas* was such as to make possible studies of flagellar structure, number, and movement. Preparations were fixed in Hollande's cupric piciformol and Schaudinn's fluid. The former fixative was always used in preparation for impregnation with activated protein silver. After fixation by Schaudinn's fluid, preparations were stained by iron hematein (0.5 per cent alcoholic solution of iron alum and 0.66 per cent alcoholic solution of hematein). Satisfactory results were also obtained by hematein staining after fixation in Hollande's fluid.

Comparative material was obtained from a grizzled grey tree-kangaroo, *Dendrolagus inustus*, in the National Zoölogical Park, Washington, D.C. A tube of egg-slant Ringer serum was inoculated from a fresh dropping and carried at room temperature for sixteen days. On arrival in Berkeley active trichomonads were present and were successfully subcultured.

The investigation has been aided by grants from the Research Committee of the University of California. Mr. Carey Baldwin, Director of the San Francisco Zoölogical Gardens, aided us in securing material. Mr. Paul Illg and Mr. M. Davis gave assistance at the National Zoölogical Park. Dr. Ross F.

Nigrelli and Dr. Leonard J. Goss aided the senior author in an attempt to obtain material from kangaroos in the Zoölogical Park of the New York Zoological Society. Most of the illustrations were made by Dr. Honigberg. Suggestions and advice on the preparation of illustrations for the paper were given by Mrs. Lois C. Taylor.

A brief report of the results of investigation of the flagellates in kangaroos in San Francisco was given at the 24th Annual Meeting of the American Society of Parasitologists (Kirby and Honigberg, 1949b).

***Trichomonas guttula* sp. nov.**

(Pl. 8, *a-d*; pl. 9, *a-d*; pl. 10, *a-h*)

DESCRIPTIVE ACCOUNT

We have given the new species name *Trichomonas guttula* to the flagellate, not having been able to identify it with any described species on the basis of published information. It is possible that among the described species of *Trichomonas* that are incompletely known one may be found of which this species is a synonym.

The size is similar to that of the common intestinal trichomonad of man. It is a small species, broadly oval in shape. The flagella are variable in length, and may be about double the length of the body. The free ends of the anterior flagella are blunt and in impregnations often appear as granules or short rods sometimes bent away from the longitudinal axis of the flagellum (pl. 8, *c*; 10, *b*). A similar structure of the free ends of the flagella was described in *Pentatrichomonas hominis* (Kirby, 1945). The group of anterior flagella originates in a single granule. There are indications in some specimens of another granule situated dorsally to this in which the costa and undulating membrane terminate (pl. 10, *e*). In most preparations there appears to be only one granule near the anterior end of the capitulum of the axostyle (pl. 8, *b*).

The costa is a slender rod and ends posteriorly at the end of the body near the place of emergence of the axostyle (pl. 8, *b*). In its anterior and posterior parts the costa tapers. The course of the costa and undulating membrane in many specimens is a slight counterclockwise spiral.

The undulating membrane is fairly broad and extends to the posterior end of the body. As demonstrated in many silver preparations, its margin is ribbonlike (pl. 8, *c*). In material in which differentiation is critical it may be observed that there appear to be two filaments in the outermost part of the membrane, one at the edge and one a short distance interior to this (pl. 8, *b*). The filament at the margin appears to continue in the posterior flagellum. The border represented at its edges by the two filaments becomes narrow where it meets the basal granule.

The free posterior flagellum is comparable in thickness to an anterior flagellum. It ends in a fine filament often 2μ or 3μ long (pl. 8, *b*, *c*). The acroneme appears distinctly in some silver impregnations.

The parabasal apparatus consists of a small body and a filament (pl. 8, *b*, *c*; 10, *h*), situated in the dorsal part of the cytosome a little to the right of

the costa and close to the nuclear membrane, but not touching the nucleus. The parabasal filament is fine, more or less straight, and 3μ or more long. Some appear to be as much as 6μ long; it is probable that often the full length has not been demonstrated.

The parabasal body is situated a short distance along the length of the filament, leaving a free part between it and the basal granule. The parabasal substance is flattened against the filament, sometimes appearing in side view as a drop hanging from it (pl. 10, *f*). The length of the parabasal body is about $1\text{--}1.4\mu$. It is more or less compressed, the width often being about $0.5\text{--}0.9\mu$. When observed in the position with the filament above, or below, it appears as a rod (pl. 8, *d*; 10, *d*). In well impregnated flagellates the center of the parabasal body is occupied by a rounded, darker area; in some this is sharply delineated as a relatively large granule (pl. 8, *b*, *c*; pl. 10, *h*). In silver impregnations the structure varies in appearance from a totally black body (pl. 10, *f*) to one whose center is black and the peripheral part clear.

The trunk of the axostyle is a slender rod about $0.2\text{--}0.3\mu$ wide in its trunk. In its anterior part the axostyle expands in a spatulate capitulum about three or four times as broad as the trunk (pl. 8, *b*). The capitulum lies on the left side of the nucleus. At the posterior end of the body the trunk of the axostyle projects for a distance that is often about 3μ . It tapers to a point but is not prolonged in a filament.

The pelta is a crescentic membrane anterior to the nucleus, close to the surface of the cytosome (pl. 10, *a*, *c-g*). It appears to be a direct extension of the capitulum of the axostyle (pl. 10, *g*). If it is not actually an extension of the capitulum, at least the membrane is applied to the anterior part of the capitulum in this region. In its dorsal part the pelta tapers and ends in a short, slender filament. The tapering part of the pelta appears to turn toward the left side, and sometimes the terminal filament bends left at a rather sharp angle. When observed from the right side the pelta may appear as a black cap (pl. 8, *c*; 10, *a*, *f*). The group of anterior flagella originates to its left near the anterior edge (pl. 8, *b*). The pelta is often blackened and consequently demonstrated clearly in silver-impregnated specimens, but it can be seen also in hematein-stained material (pl. 8, *a*).

The nucleus is broadly ovoid with its longer axis longitudinal. It is situated near the anterior end of the body. In it there is usually one endosome, which is a moderate-sized granule (pl. 8, *a*; 10, *f*). Occasionally there are two or three endosomes. As in other trichomonads, the endosome is peripheral in position and is surrounded by a clear zone. As shown in some specimens, the rest of the nucleus is filled with finely distributed chromatin granules. In many nuclei there are larger aggregations of chromatin. These larger granules are usually irregular in shape, but some may be regular enough to be endosomelike in appearance.

Dividing flagellates have been studied by dark-field illumination and so prepared by silver impregnation as to demonstrate the flagella clearly even at early stages of outgrowth. The four anterior flagella are distributed in pairs at the poles of the division figure (pl. 9, *a*). Individualization of the

two daughter mastigonts in one cytoplasmic body may be far along before a third flagellum begins to grow out at each pole (pl. 9, c). The new undulating membrane is much more rapid in its development, and reaches full size while the third flagellum is growing. Cytoplasmic division is completed while the mastigonts still have no more than three flagella. Consequently, specimens with three anterior flagella may be found among those with the complete number of four (pl. 10, a). As the next step in development, a short fourth flagellum appears in the group (pl. 10, b). That flagellum increases in length, and may come to equal the growing third flagellum before that has reached the length of the parental flagella (pl. 8, b). Specimens with all four flagella of equal length are infrequent. Probably some never grow so long as others, or division may set in before developing flagella have reached full length.

Differences in number of flagella of solitary, nondividing trichomonads of a species have been frequently reported and observed. Some counts of flagella have been inaccurate, especially those reported in older literature. These records cannot be accepted as an indication of variability. Other reports are based on the study of material in such condition that there is no assurance that the number of flagella seen is actually the same as the number originally present on the individual flagellate. In moribund material flagella may be lost. The observations here reported on the wallaroo trichomonad indicate another reason why different numbers of anterior flagella are seen—delayed outgrowth. The consequence is that flagellates may be observed with three flagella instead of four, not having developed the complete number. Precocious outgrowth is another possible reason for apparent variability, and may account for more than the normal number.

TAXONOMIC SUMMARY

Trichomonas guttula sp. nov.

Type host.—*Macropus robustus* Gould. Zoölogical Gardens, San Francisco, California. Syntype slides GP-1102: 6, 7, 9, 11.

Additional host.—*Dendrolagus inustus* Schlegel and Müller. National Zoölogical Park, Washington, D.C.

Diagnosis.—Shape broadly oval; length $8(6-11)\mu$; width $6(5-10)\mu$; four anterior flagella reaching $13-22\mu$ in length; costa a slender rod reaching the posterior end of the body; undulating membrane broad, extending the full length of the body, continued in a posterior flagellum of acroneme type $6-12\mu$ long; axostyle slender in posterior part ($0.2-0.3\mu$ wide), expanded gradually into capitulum about three or four times this breadth in approximately the anterior third, projecting from body about $2.5-3.5\mu$ and tapered to a point; pelta a large transverse crescentic membrane at apical surface, continued dorsally in a short, curved terminal filament; parabasal filament dorsal toward right, about $3-6\mu$ long; rounded, compressed parabasal body about $1-1.5\mu \times 0.5-0.9\mu$, rod-shaped in some aspects, applied to filament a micron or so from basal granule; nucleus ellipsoidal, $2.2-3\mu \times 1.7-2.2\mu$, longer axis in longitudinal direction.

COMPARISON WITH OTHER SPECIES OF TRICHOMONAS

Tanabe (1926) made a study of the comparative morphology of a number of trichomonad flagellates. Among the hosts that provided material was a kangaroo, possibly in the Zoölogical Park in Washington, D.C., or in Baltimore.

Trichomonads were found in small numbers in the feces and grew abundantly in cultures in serum-saline-citrate medium. He observed five anterior flagella, one of which lay separate from the others and originated from the same blepharoplast as the costa, whereas the group of four originated from another blepharoplast. The independent flagellum was recorded as longer than the others. The length of the flagella was not stated; as represented in the figures, the grouped flagella have a length equal to or a little greater than that of the body and the independent flagellum has about the same length. The body length was recorded as 7–10 μ . The costa and undulating membrane extend the full length of the body, with a moderately long free posterior flagellum. No information was given about the parabasal body. He named the flagellate *Pentatrichomonas macropi*. Unfortunately the type host is known only as a kangaroo.

Herman (1939) reported the results of studies of flagellates in four species of kangaroo and one wallaroo in the New York Zoological Park. A trichomonad was found in the caecum of an autopsied *Macropus robustus* and in caecal contents or feces of the other animals. It was cultured in a variety of liquid media and on blood-agar slants. The slant cultures gave the best growth when a small quantity of fecal infusion was added. Herman made studies of living flagellates, of iron-haematoxylin-stained specimens, and of Giemsa-stained preparations. The trichomonad was identified as *Pentatrichomonas macropi* Tanabe. The diagram shows the features Tanabe described—five anterior flagella, a full-length undulating membrane, and a free posterior flagellum. The independent flagellum as shown in Herman's diagram has an extraordinary length—about twice that of the body and of the grouped anterior flagella. The length of specimens in fresh material (from the caecum?) was 4.5–15 μ , whereas culture forms "tended toward an average between 7 and 10 μ ." Herman gave no information about the parabasal body.

It is not improbable that Tanabe and Herman had the same species of flagellate before them, in spite of the large discrepancy in length of the independent anterior flagellum as represented by the two authors. The characteristics they described are not sufficient to be differentially diagnostic of species. The flagellate found in San Francisco wallaroos cannot be compared with their descriptions, lacking details as they do, except for essential agreement in size and length of undulating membrane and an important difference in the absence of the independent anterior flagellum.

In view of the reports of five anterior flagella in *Pentatrichomonas macropi*, of which no type specimens are known to exist, it must be assumed for this account that the trichomonad found in kangaroos in San Francisco is a different species. The presence or absence of the independent flagellum, altogether distinct in position and activity from the group of four, must be recognized as taxonomically significant.

In its structure, aside from the presence of the independent flagellum, *Trichomonas guttula* closely resembles *Pentatrichomonas hominis*. That is at once apparent on comparison of the figures in this paper with those by Wenrich (1944) and Kirby (1945). Certain characteristics—the group of four

anterior flagella, the undulating membrane, the axostyle, and the nucleus—are common to other species of *Trichomonas* besides these two. The unique features in which there is a resemblance also are the parabasal body and the pelta. The small, rounded parabasal body of *P. hominis* is in form, size, and position much like that of *T. guttula*. There is a similar parabasal filament (Wenrich, 1944). The extension of the filament posterior to the parabasal body was not recorded by Kirby (1945), but we have seen the filament in the same material in which the body was demonstrated (fig. 1, *a*, *b*). Oc-

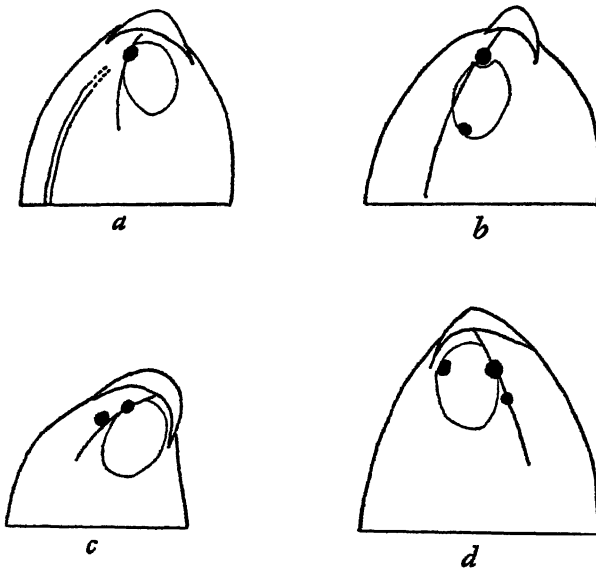


Fig. 1. *Pentatrichomonas hominis* from man. Outline of the pelta and nucleus. Parabasal apparatus consisting of a filament on which is one granule-like parabasal body (*a*, *b*) or two granules (*c*, *d*).

asionally there are two granules of parabasal substance on the filament (fig. 1, *c*, *d*).

One can scarcely escape the conclusion that so close a resemblance in every respect except the independent flagellum indicates relationship. The view that there is one species, of which some specimens have four and some five anterior flagella, may be rejected. The flagellate from wallaroos in San Francisco never has a fifth flagellum, and in any case the independent flagellum is a quite different structure from those in the group of four.

Wenrich and Saxe (1948, 1950) described *Trichomonas microti* W. & S. 1950, a flagellate of rodents which resembles *P. hominis* in many respects but, like *T. guttula*, has no independent anterior flagellum. It was found in several wild rodents: *Microtus pennsylvanicus*, *Peromyscus leucopus*, and one wild *Rattus norvegicus*. Experimental infections were established in laboratory rats, guinea pigs, and hamsters. Besides the anterior flagella, there are several structural characteristics in which *T. microti* and *P. hominis* differ. Wenrich and Saxe (1950) gave a careful and detailed comparison, and found

that the rodent trichomonad has a somewhat smaller size and more rotund form, a more markedly spiraled undulating membrane, a stouter costa, a more rectangular pelta, and a stouter axostyle. In all those features in which *T. microti* differs from *P. hominis* it appears also, from the description, to differ from *T. guttula*. The parabasal apparatus, a granule-like body and a long fibril, is similar in the three species; but we did not observe in *T. guttula* the rather stout Y-shaped strand represented by Wenrich and Saxe in figures 11, 13, and 18 of *T. microti*.

Consideration of the features of flagellates in the order Trichomonadida brings out the likelihood that the most primitive types have three anterior flagella. Increase to four is an evolutionary development which adds a new structure and may be regarded as of higher value taxonomically than quantitative differences in undulating membrane, axostyle, and parabasal body. The addition of the independent flagellum is a still more significant change; it is an added structure of new type. The origin of the *Pentatrichomonas hominis* type of flagellate might have been from a flagellate much like the *Trichomonas guttula* we describe here. The *Pentatrichomonas* type of flagellate may, as Wenrich (1944) pointed out, be found in a number of mammals as well as man, including monkeys, cats, dogs, and rats. The flagellate from a kangaroo described by Tanabe may possibly be the same thing, adding another host to the list for *P. hominis*. If that is so, in kangaroos there are both types of trichomonad, closely resembling each other except for the flagella. Further study is needed to show whether flagellates with the independent flagellum ever develop now in populations of those without the structure. More investigation is needed of the validity of the taxonomic distinctions between four- and five-flagellate trichomonads that are otherwise much alike.

***Monocercomonas* sp.**

(Pl. 10, 4)

A few specimens of *Monocercomonas* have been found on some slides made from cultures, together with large numbers of *Trichomonas* and some *Retortamonas*. We have not prepared a complete account of the flagellate nor attempted a species determination or diagnosis because of the paucity of material.

There is no doubt of the distinction between *Monocercomonas* and the accompanying *Trichomonas*. *Monocercomonas* has an elongate form and a smoother, clearer outline than that of *Trichomonas*, which in preparations is more or less irregular in shape because of the undulating membrane and the contractility. A typical specimen of *Monocercomonas* was 11μ long, with the axostyle projecting a few microns farther, and 3.6μ wide. There were three anterior flagella, the longest about 13μ long, and a recurrent flagellum about 16μ long. Other specimens had flagella 7.5μ and 7.1μ long. The anterior flagella terminate bluntly or in knobs; the recurrent flagellum ends in a fine filament. The recurrent flagellum has about the same thickness as an anterior flagellum. There can be no confusion with a possibly detached margin of the undulating membrane of *Trichomonas*; a detachment not observed in the

material examined. The margin of the membrane has a ribbonlike or double-filament form, and there is an abrupt difference in size between that margin and the free posterior flagellum.

The parabasal body is conspicuously different from that of *Trichomonas*. It appears in protargol preparations as a stout rod about 1.5μ long, which lies mostly against the anterior contour of the nucleus, and comes in direct contact with the basal granule. No filament separating the parabasal body from the granule, as in *Trichomonas guttula*, has been seen in *Monocercomonas* sp.

***Retortamonas mitrula* sp. nov.**

(Pl. 11, *a-m*; fig. 2)

DESCRIPTIVE ACCOUNT

The flagellate is small, even for a species of *Retortamonas*, and is somewhat longer than broad. Living specimens are longer in relation to the width than are fixed ones; some measured about $10\mu \times 6\mu$. The anterior end is broadly rounded and the posterior end is rounded, or sometimes, although not narrowed sharply, comes to a point. When a caudal process is present it is very short, in contrast to the relatively long process in the type species of the genus, *R. grillotalpae*. On one surface in the anterior section of the body the rounded contour is broken by a flattened or depressed area, which is the place of the cytostome.

The two flagella leave the body anterolaterally and are about equal to one another in length and thickness. In dark-field preparations the recurrent flagellum often appeared somewhat more slender than the other (fig. 2). Their length in many fixed specimens is nearly equal to that of the body or at most only a few microns shorter (pl. 11, *a*). Living specimens 10μ long studied in dark field had anterior flagella about 5μ long. Both flagella are of the acroneme or Peitschengeissel type, terminating in a fine filament. The filament is frequently somewhat less than a micron in length, though sometimes longer. The most anterior part of the recurrent flagellum has a more or less fixed position in relation to the anterior part of the cytostome. That flagellum is seldom turned forward to project beyond the anterior end of the body.

In dark-field preparations of living flagellates in culture it was possible to observe the form and activity of the flagella, the outline of the cytostome, the ingestion of bacilli, and the movements of bacilli in the cytoplasm. The anterior flagellum originates anteroventrally and sweeps from side to side. The recurrent flagellum emerges within the anterior part of the cytostome and its first part passes longitudinally within and along the cytostome. Its activity is different from that of the anterior flagellum. It does not change its position much, but undergoes a rapid undulatory movement. Stout rod-shaped bacteria, which were abundant in the medium, were repeatedly seen to enter the body at the cytostome. In many flagellates, bacilli in the cytoplasm were seen to move fairly rapidly from end to end of the body, presumably indicating streaming movements of the cytoplasm (fig. 2). The posterior end

of the body is rounded or, more often, broadly tapered as in figure 2; infrequently there is a short caudal process.

The cytostomal region is broadly oval in form, with its anterior margin a short distance posterior to the anterior end of the body (pl. 11, *a*, *b*). It extends posteriorly for about a quarter to a third of the body length. Its

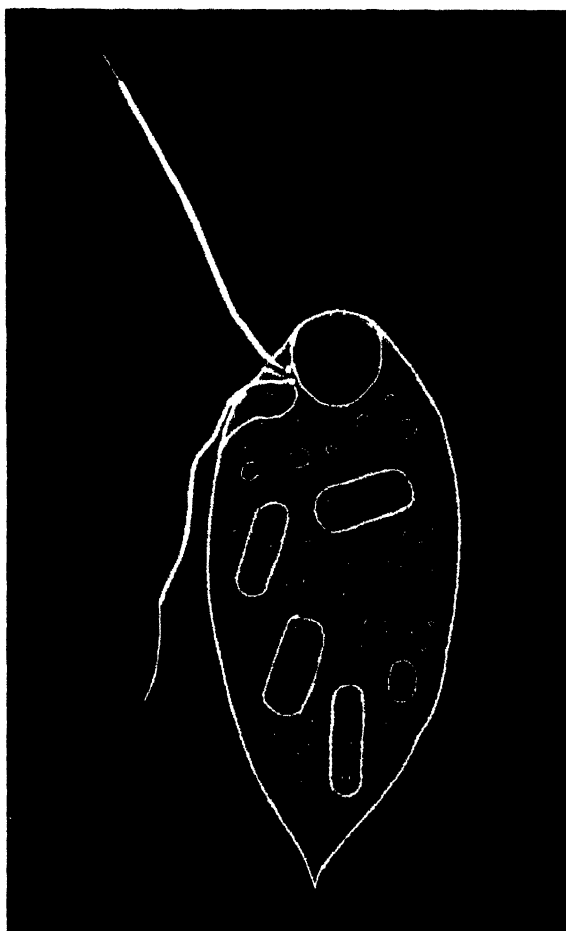


Fig. 2. *Retortamonas mitrula* from *Macropus robustus*. Freehand drawing made from living flagellates observed by dark-field illumination. Outline of cytostome, nucleus, basal granules, and acroneme flagella; ingested bacteria in cytoplasm.

edges do not project noticeably beyond the general body contour. The anterior border of the depression is impregnated in protargol preparations, appearing as a curved membrane whose form may be projected in triangular (pl. 11, *a-c*, *e-g*) or trapezoidal (pl. 11, *d*, *h*) form. The usual direction of the flat surface of the membrane is more or less dorsoventral. From its edge at the margin of the body to its opposite edge, or apex, nearer the longitudinal axis,

it measures about one micron. This measurement is evidently that of the depth of the cytostomal depression at its anterior part; more posteriorly it is shallower. From the sides of the anterior impregnating membranelike region, narrower lateral structures continue to border the sides of the cytostomal region (pl. 11, *g, i*). It is likely that the arched membranous structure constitutes a differentiated marginal cytoplasm bordering the cytostome, rather than a discrete structural element. The entrance to the body appears to be restricted to the anterior part of the cytostomal region.

The marginal fibril which has been referred to in all descriptions of flagellates of this genus (which includes *Embadomonas*) appears more consistently as a discrete structure. It can readily be demonstrated by iron-haematoxylin, whereas the membranous structure described above has been seen clearly only in silver-impregnated material. The fibril lies at the margin of the cytostomal region in a smooth curve, more or less in U-form with the arms directed posteriorly (pl. 11, *c*). It impregnates more deeply than the membranous structure at the edge of which it lies. The two longitudinally directed parts of the fibril are almost equal in length and usually appear to be equal in thickness. Often, however, the left part of the fibril impregnates less than the right part, and so appears to be more slender and shorter. The cytostomal fibril is superficially placed throughout its length. Neither of the posterior parts of the fibril extends into deeper levels in the cytoplasm, as does one part in species of *Chilomastix*.

The flagella originate from two basal granules in the cytoplasm just above the inner edge of the anterior part of the cytostome, and against the membrane of the nucleus (pl. 11, *a*). The granule at the end of the recurrent flagellum is placed at the inner margin of the cytostomal depression; the flagellum extends free in that depression before emerging to lie outside of the body. The granule that gives origin to the anterior flagellum is situated anterior to the other granule and close to it. The flagellum extends entirely free of the body anterior to the region of the cytostomal depression. The two flagella thus diverge markedly at their origin, and are separate from one another for all their length.

The nucleus is spherical and, with a diameter of 1–2 μ , is relatively large in size. Its position in the body is as far anterior as possible, its edge lying at the anterior margin of the body. Interior to the nuclear membrane is a layer of chromatic substance which appears in optical section as a more or less continuous narrow ring (pl. 11, *a, b*). A relatively large endosome, which sometimes is dispersed in several granules, may be demonstrated in the central part of the nucleus. Protargol impregnations sometimes show the following structure, which is considered to be typical of *Retortamonas*: a peripheral ring, a relatively large endosome, and a clear zone between the two (pl. 11, *b*). But often the nucleoplasm impregnates uniformly, dark or light, or the interior of the nucleus is clear. Protargol impregnation is a capricious technique in demonstrating structure in the nucleus. It is perhaps, not to be expected that a silver-impregnation technique should show as much of the interior nuclear structure as the Bodian method often does.

Some dividing flagellates were studied in silver-impregnated preparations. These contained at each pole a fibril-bordered differentiated cytostomal region and two flagella. In the specimen represented by plate 11, *j*, one flagellum at each pole is markedly shorter than the other. The shorter is probably newly outgrown, the other a parent flagellum, the two original flagella having been distributed one to each daughter mastigont. We have no evidence whether the cytostomal region and fibril are retained or resorbed. The small and equal size of the cytostomal regions shown in the figure suggests that both are neoformed, the parent region having dedifferentiated.

Double individuals were common in some preparations, occasionally constituting as much as 18 per cent of the population (pl. 11, *k*, *l*). In these specimens there were two nuclei and two complete, fully reorganized cytostomal areas and sets of flagella. Three triple individuals were found, each having three nuclei and three complete sets of organelles (pl. 11, *m*).

Double individuals are not infrequent in other species of *Retortamonas*, and triple individuals also occur. Bishop (1931) found that many *Embado- monas dobelli* in culture had duplicated organelles, and she referred to other observations of double forms in species of the genus. Dobell (1935) stated that double individuals of *Embado- monas intestinalis* often occur in feces and in cultures. The flagellates remain in the double condition for long periods without division. Bishop found individuals of *E. dobelli* with three nuclei and three sets of organelles to be rare.

TAXONOMIC SUMMARY

Retortamonas mitrula sp. nov.

Type host.—*Macropus robustus* Gould. Zoölogical Gardens, San Francisco, California. Syntype slides GP-1239: 17, 18, 20, 21.

Diagnosis.—Rounded anteriorly, usually narrowed posteriorly, caudal process absent or very short; length of fixed specimens from culture $5.5(3.5-7.5)\mu$, living specimens up to about 10μ long; width $4(3-5.5)\mu$; flagella $5.5(4-7)\mu$ long, the two about equal in length, both terminating in filaments about 1μ long; basal granules of flagella close together and adjacent to anteroventral surface of nuclear membrane; cytostomal region broadly oval, beginning near anterior end, length about a quarter to a third of the body length; cytostomal fibril single, rounding the anterior edge of the cytostome, with two approximately equal arms along the sides, anteriorly bordering a curved triangular or trapezoidal membrane that bounds the anterior surface of the cytostomal depression; nucleus spherical, $1-2\mu$ in diameter, anterior edge at the rounded anterior margin of the body, with large central endosome and layer of chromatic material under the nuclear membrane.

COMPARISON WITH OTHER SPECIES OF RETORTAMONAS

Species of *Retortamonas* have been recorded from various insects, amphibia, reptiles, and mammals. Wenrich (1932) listed fifteen species. Among them are doubtless several synonyms. Two other species from insects are *Retortamonas termitis* (Kirby, 1932), host *Amitermes beaumonti*, and *R. wenrichi* Stabler, 1944, host *Gryllotalpa hexadactyla*. Wenyon (1926) reported having found an *Embado- monas* in a tortoise in the Zoölogical Gardens in London. Knowles and Das Gupta (1931) described as *Embado- monas ruminantium* a

flagellate cultured from the feces of eight consecutively examined bulls, *Bos taurus*, in India. It is certain that further exploration, particularly of insects and mammals, will increase the list of hosts of *Retortamonas*. If the concept of species adopted by some previous investigators of flagellates of this genus continues to be followed, there will be almost as many more species as there are newly discovered hosts. It is to be hoped, however, that a sounder basis for differentiating species will be worked out. Recognizable differences in the characteristics we can use in diagnosis should be established when a new species is made.

Species of *Retortamonas* have been differentiated from one another, when the taxonomist has considered it necessary to look for more than a host difference, on the basis of size and shape of the trophic stage and cyst, the relative length and stoutness of the flagella, and the characteristics of the cytostome.

In that it does not exceed 7.5μ in length, *Retortamonas mitrula* is small compared to some other species, which reach a length of 20μ or even more. Its maximum is close to or below the minimum in such large species as *R. grilotalpae*, *R. alexeieffi*, and *R. caudacus* from insects, and *R. cuniculi* from mammals. A long caudal process is particularly characteristic of species from beetle grubs and mole crickets; that of *R. wenrichi*, according to Stabler (1944), may be 58μ in length, on a body whose length is not more than 19μ . In insects there are also small species, such as *R. agilis*, $4-11\mu$ long, and *R. blattae*, $6-9\mu$ long, which do not have long caudal processes. Nevertheless, an intestinal flagellate of an insect can reasonably be excluded from possible identification with an intestinal flagellate of a mammal, at least unless there is indisputable contrary evidence.

In accounts of species of *Retortamonas*, reports of differences in the thickness and length of the two flagella of an individual flagellate are frequent. The recurrent flagellum of *R. agilis*, according to Mackinnon (1915) and Ludwig (1946), is shorter and more delicate than the other. That of *R. blattae*, according to Bishop (1931), is much more slender and smaller than the free anterior flagellum. In certain other species the cytostomal flagellum is described as the thicker one, and may or may not be shorter than the other. Such a recurrent flagellum characterizes *R. dobelli*, according to Bishop (1931); *R. bradyi*, *R. caviae*, and *R. ovis*, according to Hegner and Schumaker (1928); and *R. cuniculi*, according to Collier and Boeck (1926). It has usually been stated to be characteristic of *R. intestinalis* of man (Wenyon and O'Connor, 1917a,b; Hogue, 1921; Dobell and O'Connor, 1921; Jepps, 1923; Wenyon, 1926). Most writers have accepted the reported difference without question, though it had been observed only in iron-haematoxylin preparations, in which the nature of the flagella can usually be demonstrated only with difficulty. When Hogue (1933, 1936) used dark-field illumination to study retortamonads from man, and found the two flagella to be, in general, equal in thickness and length, she considered it probable that a varietal difference from the originally described form was indicated. Faust (1922) reported the flagella to be equal in length and thickness in *Embdomonas sinensis* in man.

The flagella of *Retortamonas mitrula* are equal in thickness and length, as can be shown by dark-field observation and by silver impregnation. In that the flagella are equal, the flagellate may seem to differ from others that have been described from mammals, excepting the forms of the intestinal retortamonad of man that were described by Hogue and by Faust. Silver impregnation has, however, been used in study of no other species than the one we describe here, and only Hogue reported using dark-field illumination.

A satisfactory technique for demonstrating flagella is Giemsa staining. Though Knowles and Das Gupta (1931) stated that the anterior flagellum of *R. ruminantium* is longer and thinner than the intracytostomic one, their figures of Giemsa-stained specimens represent the flagella as more or less equal in length and thickness. Because of the possibility of incomplete demonstration by commonly used techniques, of mechanically produced staining differences, and even of the influence of preconceived concepts, it is unsafe to make comparisons for taxonomic purposes in *Retortamonas* from descriptions alone. More than a few protozoölogists have wrongly named new species and even genera because their observations failed to coincide with published descriptions of what in reality was the same species they had before them.

The differentiated region of the body in which the first part of the recurrent flagellum lies is the structure that especially characterizes the genus. This has been termed the cytostome (Mackinnon, 1911), and the oral pouch (Wenrich, 1932). Various authors have reported seeing bacilli and other food particles entering the cytostome, so there seems to be no doubt about its function. The borders of the cytostome are in some species reported to be raised in lips. The characterization of the genus *Embadomonas* Mackinnon, 1911, by Chalmers and Pekkola (1918) is: "With one anterior and one posterior flagellum which is generally cytostomic and with a large cytostome with siderophilous, often folded border." In some descriptions the designation has been that of siderophilous lips (Mackinnon, 1915), in others it has been stated that a single circuitous fiber extends around the whole cytostome (Collier and Boeck, 1926). More recent critical studies have brought out the absence of a connecting fibril around the posterior margin (Bishop, 1931, in *E. dobelli*; Wenrich, 1932, in *R. grylloalpa*; Ludwig, 1946, in *R. alexeieffi*; and others). Several writers have reported two fibrils, one on each side, though without showing that these are actually separate anteriorly. Wenrich (1932) and Stabler (1944) believed that the fibrils correspond to the "parabasal-fiber" and "parastyle" which had been so named in *Chilomastix* (Kofoid and Swezy, 1920). In *Retortamonas mitrula*, however, there is a single fibril rounding the anterior border and extended posteriorly on each side, a fibril not directly attached to the basal granules. The structure is comparable to that described by Kirby and Honigberg (1949a) in *Chilomastix magna*. Nie (1948) showed a similar cytostomal border in *Chilomastix intestinalis* and other species (cf. his pl. 1, figs. 1, 6, 8 and our pl. 30, b, d). Though Nie insisted on the interpretation of two cytostomal fibrils, that seems not necessarily to follow from his carefully made illustrations and some of his descriptive accounts.

On the basis of published descriptions, *Retortamonas mitrula* from *Macropus robustus* could not be identified by us with any existing species. But neither can it be certain that it may not be identical with some described species, especially one from a mammalian host. Not even *Retortamonas intestinalis* has been completely enough described so that a confident comparison can be made. Furthermore, cysts of the kangaroo flagellate have not been found. One must decide between leaving it unnamed, on the supposition that it may eventually be found to belong to an already known species, or supplying a name that may eventually fall into synonymy. The latter course is the one we have adopted, though not without hesitation. It provides a more definite basis of availability to this record.

DISCUSSION OF THE GENERIC NAME RETORTAMONAS

Wenrich (1931, 1932) gave reasons for believing that the species of *Embadomonas* Mackinnon, 1911 (mt. *E. agilis* Mackinnon, 1911) are congeneric with *Retortamonas grillotalpae* Grassi, 1879, the type species of *Retortamonas* Grassi, 1879. In the latter paper Wenrich listed fifteen species of *Retortamonas*, thirteen of which had previously been placed in *Embadomonas* or in *Waskia*. Wenrich's taxonomic conclusions in this matter have been widely but not universally accepted. Objections were published by Bishop (1934) and Dobell (1935), and Wenrich's position was defended by Corradetti (1937). Bishop's refusal to accept the change was the result of her opinion that Grassi's 1882 figures and description of *Plagiomonas Grillotalpae* (his new name for his older *Retortamonas grillotalpae*) do not sufficiently resemble *Embadomonas*, and especially of her knowledge that Grassi in 1888 defined *Plagiomonas* in terms different from those of his earlier accounts. She concluded from his later definition that *Plagiomonas* has three flagella, whereas *Embadomonas* has two. Dobell's objections were based on the same findings. He considered Wenrich's evidence insufficient to justify the nomenclatural change, and stated that "these genera, as defined by their authors, have, indeed, almost nothing in common."

Wenrich (1932) gave a good description of the flagellate in *Gryllotalpa gryllotalpa* from New Jersey which he regarded as *Retortamonas grillotalpae* Grassi.¹ Wenrich believed that the flagellate he then described belonged to the same genus as the caddis-worm flagellate that Mackinnon (1911) named *Embadomonas agilis*. There is no escaping that conclusion when Wenrich's description of the mole-cricket flagellate is compared with that of *E. agilis* by Mackinnon (1915). Furthermore, Wenrich himself examined specimens

¹ Grassi (1879, 1882) wrote the name *Retortamonas grillotalpae*, capitalizing the initial letter of the trivial name in the latter paper and giving it as a synonym of *Plagiomonas Grillotalpae*. In 1882-83 he wrote the name *Retortamonas Gryllotalpae* as a synonym of *Plagiomonas Gryllotalpae*. Stiles (1902) pointed out that the generic name *Plagiomonas* Grassi is not valid, as Grassi (1879) had already proposed *Retortamonas* with the same species (*gryllotalpae*) as only, hence type species. In this statement by Stiles there is no original contribution to the nomenclature of the species name that warrants including his name in the authorship, as did Wenrich (1932) in writing "*Retortamonas gryllotalpae* Stiles, 1902," and "*Retortamonas gryllotalpae* (Grassi, 1879) Stiles, 1902." The name *Retortamonas grillotalpae* Grassi, 1879, need not be altered. But even if the altered spelling of the trivial name is considered necessary and should be recognized as warranting new authorship, that authorship should be Grassi, 1882, not Stiles, 1902.

of the caddis-worm flagellate. So it is evident that *Gryllotalpa gryllotalpa* either contains both a species of *Retortamonas* and one of *Embadomonas*, or the flagellate Wenrich described is a species of *Retortamonas*.

Wenrich (1932) and Corradetti (1937) reported three flagellates in the mole-cricket *Gryllotalpa gryllotalpa* in New Jersey and in Italy. One is a species of *Hexamastix*, probably *H. gryllotalpae* Grassé, 1926. It seems that Grassi did not name that flagellate, though he may have referred to it when he wrote (1882, p. 154) of a form with a caudal extremity almost as long as the body to double that length. Grassé figured (1926, pl. 19, fig. 309) *H. gryllotalpae* with a very long caudal projection. Another flagellate is probably the one named by Grassi (1879) *Schedoacercomonas grillotalpae*; Wenrich (1932) called this *Monocercomonas gryllotalpae* and we consider it to belong to the genus *Monocercomonoides* Travis, 1932. The third flagellate is the one Wenrich identified as *Retortamonas grillotalpae* Grassi, 1879.

Grassi (1879) characterized *R. grillotalpae* in these words: "Grossolanamente somiglia ad una storta; si scorge un'apertura boccale (?); non rilevai che due flagelli." In 1882 he wrote of this again and illustrated the curved anterior form, the clear area in the anterior part (which evidently represented the cytostome), the short, gradually narrowing (or rodlike) caudal extremity, and the two long flagella, one directed anteriorly and the other posteriorly. All these things apply to the flagellate Wenrich described, and that author prepared specimens by the same technique of iodine staining that Grassi used, and gave comparative figures to show the close resemblance in the few but highly significant characteristics revealed. Certainly we must admit that as far as can be judged from Grassi's description the flagellates have a good deal in common.

Bishop (1934) pointed out the importance of the fact, not considered by Wenrich, that Grassi in 1888 defined *Plagiomonas* (*Retortamonas*) as having three flagella, two anterior and one posterior. His definition (1888, p. 9) is "*Plagiomonas*: Due flagelli anteriori, diretti anteriormente, ed uno posteriore (caudale)." It should be compared with his earlier statement (1882, p. 161) about the flagella of *Plagiomonas*, "L'estremità anteriore porta due (?) flagelli lunghi più del corpo; l'uno è rivolto in avanti e l'altro indietro." A different interpretation from that made by Bishop is possible. In the same paper Grassi gave the following definition of *Bodo* Ehrbg. (Sin. *Heteromita* Duj.): "Due flagelli anteriori, uno diretto anteriormente e l'altro posteriormente: nessuno posteriore." In the first part of his definition Grassi accounted for the anteriorly directed flagellum and the recurrent flagellum, which he did not consider to be a posterior flagellum. In his definition of *Dicercomonas* (actually *Hexamitus*),—"Quattro flagelli anteriori, due flagelli posteriori,"—Grassi obviously thought of a posterior flagellum as having direct origin from the posterior end of the body. In the flagellates of the group to which *Retortamonas* (= *Plagiomonas*) belongs there is no posterior flagellum in that sense. Indeed, no flagellate is known to exist in which the characteristics indicated in the mole-cricket form are combined with a caudal flagellum. The 1888 statement by Grassi may be based on his having at that time (though

not earlier) thought of the slender caudal process shown in his 1882 figures (which as Wenrich, 1932, showed is characteristic of the species) as a posterior flagellum. In *Retortamonas hexadactyla* Stabler, 1944, from *Gryllotalpa hexadactyla*, the tenuous caudal process is ordinarily longer than the body, and may be a filament several times as long.

Corradetti (1937), who reviewed in detail the matters that we are discussing here, was the first to suggest that the caudal process was the basis for Grassi's 1888 statement about the third, caudal flagellum. He was of the opinion that Grassi's statement was due to a mistaken interpretation, without new observations, of studies made several years before.

We may reasonably consider it to be true that the only actual flagella Grassi (1888) recognized in *Plagiomonas* were the two anterior flagella. In his statement that the two anterior flagella are directed anteriorly he is in disagreement with his own statement of 1882, with his figures, and with the probabilities among flagellate inhabitants of insects. Grassi must have been wrong in one of his descriptions. Corradetti (1937) doubted the validity of the later, conflicting statement. There seems to us to be sounder reason, as Corradetti maintained, for accepting the longer, more detailed, and illustrated, 1882 account than for rejecting it in favor of the brief, improbable statement of 1888, which is accompanied by obscure interpretations and is evidently not based on any further studies of the material.

The conclusion of Dobell (1939) that O. F. Müller's *Cercaria tenax*, observed in the course of examination of material from the human mouth, was the trichomonad of the mouth, which therefore should be named *Trichomonas tenax*, is likewise based solely on probability. The few characteristics Müller described could have been those of the trichomonad, but could also apply to a large number of other species and genera of protozoa. The only possibility of determining what Müller had comes from knowledge of the habitat of his organism. Knowing that the material examined came from the human mouth, Dobell rightly considered what organisms from that environment Müller might have seen. Among the normal inhabitants of the mouth, only the one trichomonad, he concluded, could have been so described; the likelihood that Müller could have seen anything else is slight. Dobell remarked that "the indication is no less certain than that for dozens of other Müllerian genera and species which are nowadays accepted universally." We are fully in agreement with Dobell's conclusions in this matter of *Trichomonas tenax*. If we were to insist on complete and unexceptionable evidence of identity of forms we can observe with those described by early protozoölogists who left no type specimens, we could not use many of the older generic names that are now employed, and would have to reject as undeterminable most of the species names.

There is another aspect of this problem. Neither *Retortamonas* Grassi, 1879, nor *Embadomonas* Mackinnon, 1911, has yet been placed in the Official List of Generic Names in Zoölogy. Since it appears that *Embadomonas* is a synonym of *Retortamonas*, it can be retained only by use of the plenary powers of the International Commission of Zoölogical Nomenclature to sup-

press Grassi's name. A request to the Commission so to act would be based upon the supposed advantage of continuing the use of a more familiar generic name, particularly since it involves the name of a flagellate that occurs in man. The flagellate of man is, however, one primarily of zoölogical, not medical, interest. It was first known as *Waskia intestinalis*, though that name was soon abandoned in favor of *Embadomonas intestinalis*. Only thirteen years later Wenrich pointed out that *Retortamonas* is the correct name to use, and a few years later Corradetti supported his conclusions, as a result of original studies. In the last twenty years, *Retortamonas* has been adopted by several authors. There would be no advantage, other than satisfying certain individual preferences, in calling upon the Commission to use the plenary power. Such a request should be made only when it is clearly in the interest of stability in nomenclature. Stability is not necessarily justifiably promoted by attempting to fix for centuries the results of usage, based on incomplete information, in a period of only twenty years or so.

RELATIONSHIP BETWEEN RETORTAMONAS AND CHILOMASTIX

The relationship between *Retortamonas* and *Chilomastix* has been discussed by several authors (Mackinnon, 1915; Alexeieff, 1912, 1917; Wenrich, 1931, 1932; Kirby and Honigberg, 1949c). Alexeieff (1917) suggested the grouping of the two flagellate genera in a new family Embadomonadidae. Wenrich (1931, 1932) proposed the new family Retortamonadidae, based on the correct name of the genus. The similarity between these two groups of flagellates is much greater than the resemblance of either one to any other known flagellate. The type of cytostomal depression is common in structure and location, and occurs in no other genus. The fibril rounds the anterior margin of the cytostome and continues posteriorly along each of the sides. The nucleus is situated anteriorly, is spherical, and has an essentially comparable structure. The structure of the nucleus does not here necessarily indicate a relationship; that is based primarily on the distinctive cytosomal organization. In both, a granule near the anterior margin of the cytostome gives origin to a recurrent flagellum, the first part of which, at least, lies in the opening of the cytostome. One anterior flagellum originates from a nearby granule in *Retortamonas*; in *Chilomastix* there are two other separated granules that give rise to two other flagella. There is no parabasal body and no axostyle.

Kofoed and Swezy (1920) discussed the apparent relationship between *Chilomastix* and *Giardia*, stating that the Hexamitidae, including *Giardia*, must be regarded as an offshoot from *Chilomastix* or its near relations. That position cannot be defended. The ventral depression of *Giardia* has nothing in common with the cytostome of *Chilomastix*. Neither the fibrils bordering the depression nor the cytostome indicate any relationship. The posterior flagella of *Giardia*, with the so-called axostyles constituted by their intracytoplasmic parts, must be comparable to a recurrent flagellum rather than to the part of the cytostomal fibril of *Chilomastix* improperly designated the "parastyle."

When the customary criteria for the separation of orders in the nonpigmented Flagellata are adopted, *Retortamonas* goes into one order and *Chilomastix* into another. The Protomonadida, defined as having one to two flagella, takes in the former genus; the Polymastigida, defined as having three to eight flagella, receives *Chilomastix*. If we are correct in believing that there is a fundamental relationship between *Retortamonas* and *Chilomastix*, that is a good illustration of the artificiality of establishing these orders of Flagellata solely on the basis of the number of flagella.

SUMMARY

Three species of flagellates were cultured from feces of *Macropus robustus* kept in the Zoölogical Gardens in San Francisco. Two of these are described as new species, *Trichomonas guttula* and *Retortamonas mitrula*. A species of *Monocercomonas* was found in small numbers.

Trichomonas guttula was also found in cultures from feces of *Dendrolagus inustus* in the National Zoölogical Park, Washington, D.C.

Trichomonas guttula has four anterior flagella and no fifth anterior flagellum as was described in *Pentatrichomonas macropi*. The parabasal apparatus consists of a long filament along the anterior part of which a small, rounded or discoid parabasal body is applied. In pelta, parabasal body, axostyle, and undulating membrane, *T. guttula* has much resemblance to *P. hominis*.

Retortamonas mitrula has two equal-sized flagella of acroneme type, and a cytostome at the margin of which is a continuous fibril passing around the anterior edge. A membranous differentiation borders the anterior surface of the cytostomal depression. A relationship to *Chilomastix* is indicated primarily by the nature of the cytostomal structures. The family Retortamonadidae seems to be justified.

A review is given of the nomenclatural status of the genus *Retortamonas*, and it is concluded that the name properly replaces *Embadomonas*, which is a synonym thereof.

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PLATES

All figures based on camera lucida outlines.

Abbreviations for methods of preparation: HP., Hollande's cupric piciformol—Bodian's protargol; SH., Schaudinn's fluid—Iron hematein (0.5 per cent alcoholic solution iron alum and 0.66 per cent alcoholic solution of hematein).

PLATE 8

Trichomonas guttula sp. nov.

Magnification, $\times 3870$.

a. Mastigont organelles originate in a single large granule which is partly covered by the crescentic pelta; undulating membrane bordered by a marginal filament which continues in the posterior flagellum; axostyle with spatulate capitulum on the underside of the nucleus, parabasal body not shown by this technique. Length 8.1μ . SH.

b. Flagella impregnated by silver appear much heavier than in *a*, end in knobs or short rods, differ in length; pelta lies under the proximal part of group of flagella; margin of undulating membrane with two filaments; posterior acroneme flagellum; parabasal apparatus with long filament and small rounded body with inner granule; axostyle expanded in anterior part. Length 9.5μ . HP.

c. Specimen similar to *b* from the right side; the more heavily impregnated membrane margin appears as a ribbon. Length 9.3μ . HP.

d. Antapical view showing the pelta, undulating membrane, nucleus, origin of flagella, and parabasal body which in this position appears as a rod. Longest diameter 7.4μ . HP.

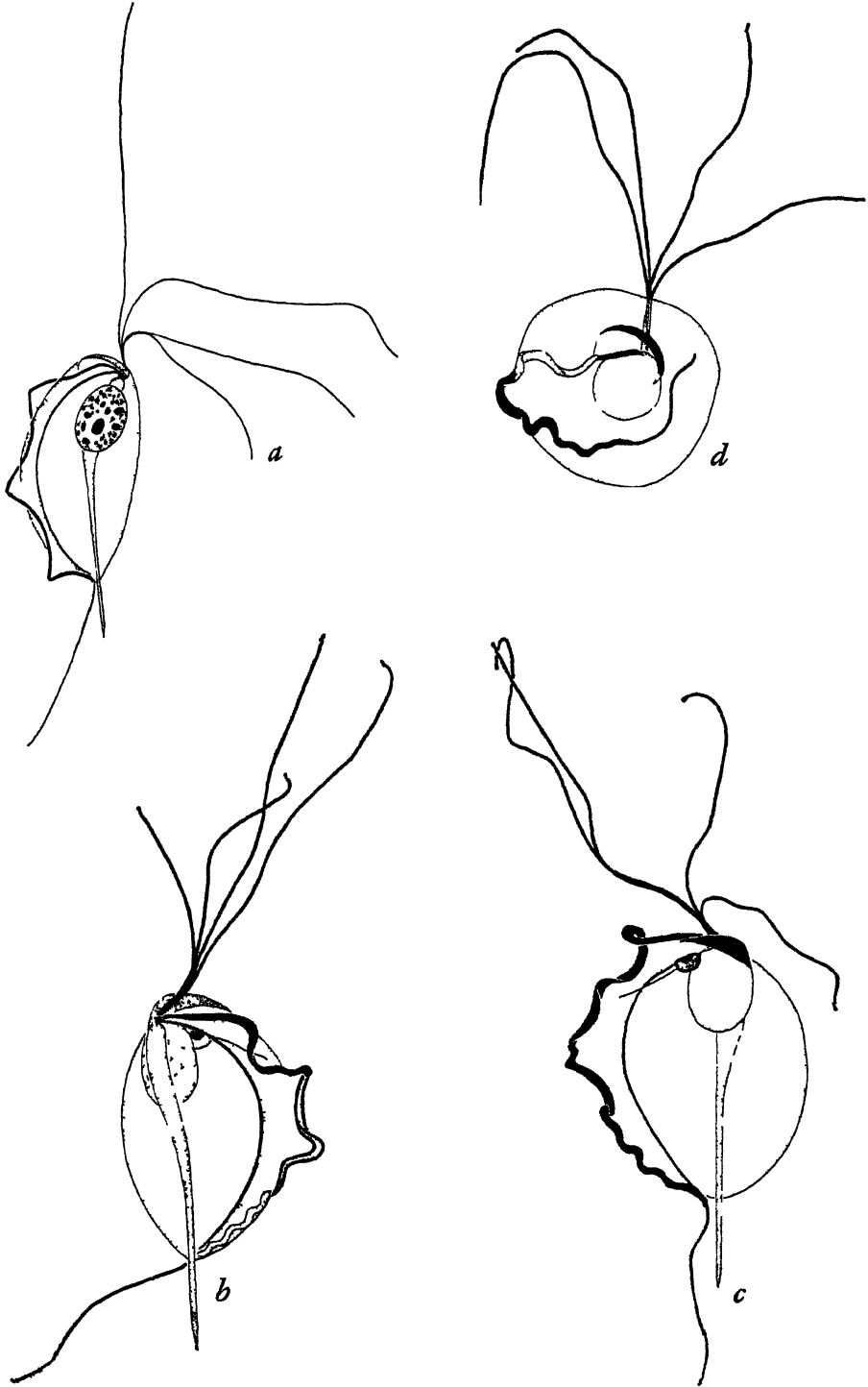


PLATE 9

Trichomonas guttula sp. nov.

Magnification, $\times 3870$. All HP.

a. Early division with the original flagella distributed in pairs, one pair at each pole. Undulating membranes omitted. Longest diameter 6.9μ .

b. Late telophase with two original flagella at each pole, no new ones yet growing out, two well-developed undulating membranes. Longest diameter 7.1μ .

c. Division stage later than *b*, showing parabasal bodies and filaments, costas and undulating membranes, primordia of new axostyle, short third flagellum growing out in each mastigont. Longest diameter 8.8μ .

d. Cytosome not yet divided, third flagellum longer than in *c*. Longest diameter 9.8μ .

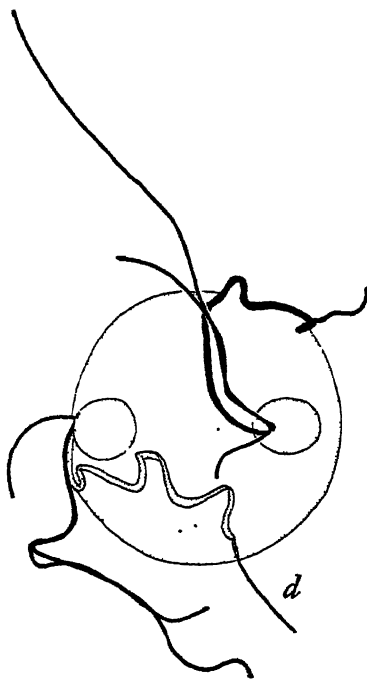
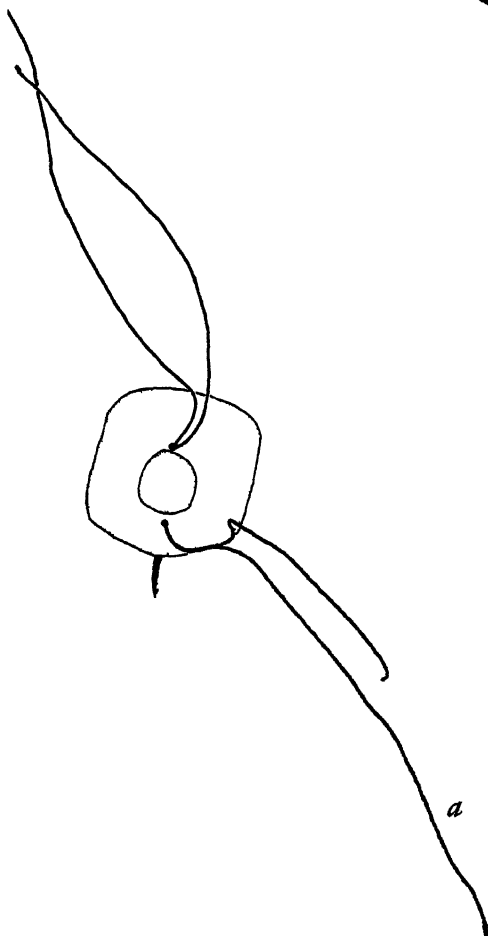
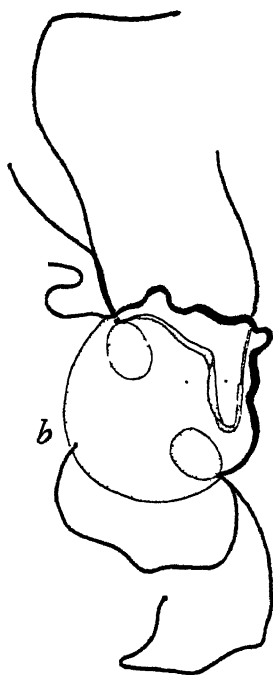
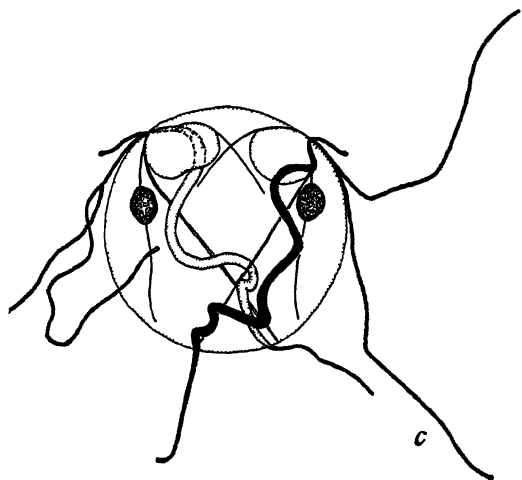


PLATE 10

a-h. *Trichomonas guttula* sp. nov.

i. *Monocercomonas* sp.

Magnification, a-h, $\times 3870$; i, $\times 3370$.

a. Flagellate after division of the cytosome, in which the fourth flagellum has not yet grown out. In this trichomonad, division occurs when there are as yet only three anterior flagella in each mastigont, and consequently many three-flagellate solitary forms may be found.

b. Outgrowth of fourth anterior flagellum.

c. Crescentic pelta continuous with the anterior part of the capitulum of the axostyle proper.

d. Pelta continuous with anterior part of axostyle, as in c; parabasal body appearing in this aspect as a rod.

e. Origin of mastigont structures in two granules, one for the group of anterior flagella, other for costa and undulating membrane.

f. Pelta anterior to nucleus; discoid parabasal body applied to parabasal filament.

g. Flagellar granule met by mastigont structures, including parabasal filament; four anterior flagella.

h. Parabasal apparatus with long parabasal filament and rounded parabasal body with deeply impregnated inner mass. This is the typical characteristic of the parabasal body.

i. Diagram of *Monocercomonas* sp. Three anterior flagella ending in knobs; long, acroneme type recurrent flagellum; curved pelta at anterior edge of body; elongate rod-shaped parabasal body directly applied to basal granule, instead of attached by long filament as in *Trichomonas guttula*. Length 7.1μ ; projecting axostyle 2.3μ .

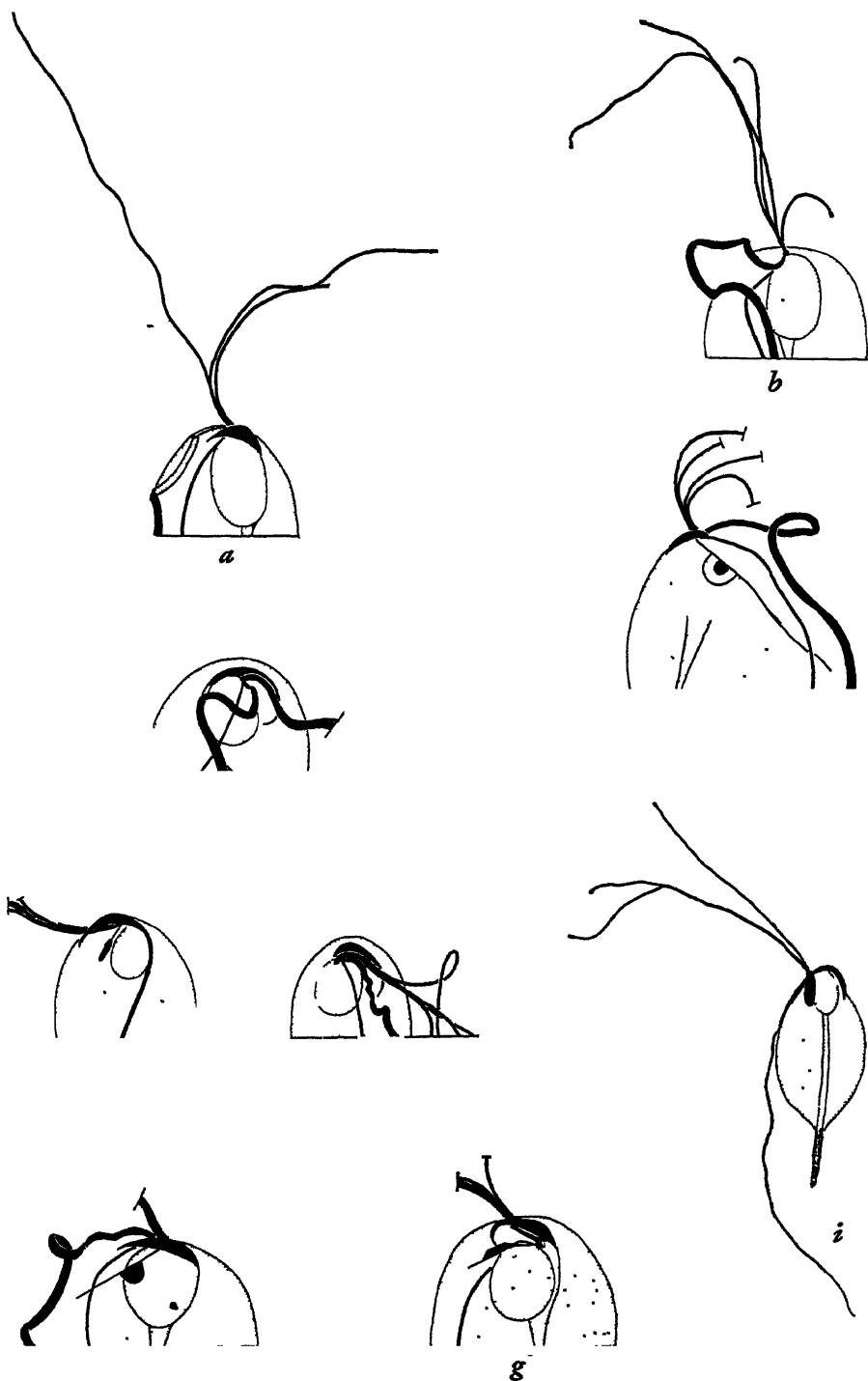


PLATE 11

Retortamonas mitrula sp. nov.

Magnification, $\times 5100$. All IIP.

a. Anteriorly placed nucleus with central group of granules and dark marginal layer; each flagellum ends in a granule that lies against the nucleus; both flagella of acroneme type; proximal part of recurrent flagellum lying in cytostomal depression, which is bordered by fibril. Length 5.6μ .

b. Cytostomal fibril and triangular impregnated area at anterior part of cytostome. Length 5.1μ .

c. Ventral view showing cytostomal structures and recurrent flagellum emerging from under the anterior membrane. Length 4.7μ .

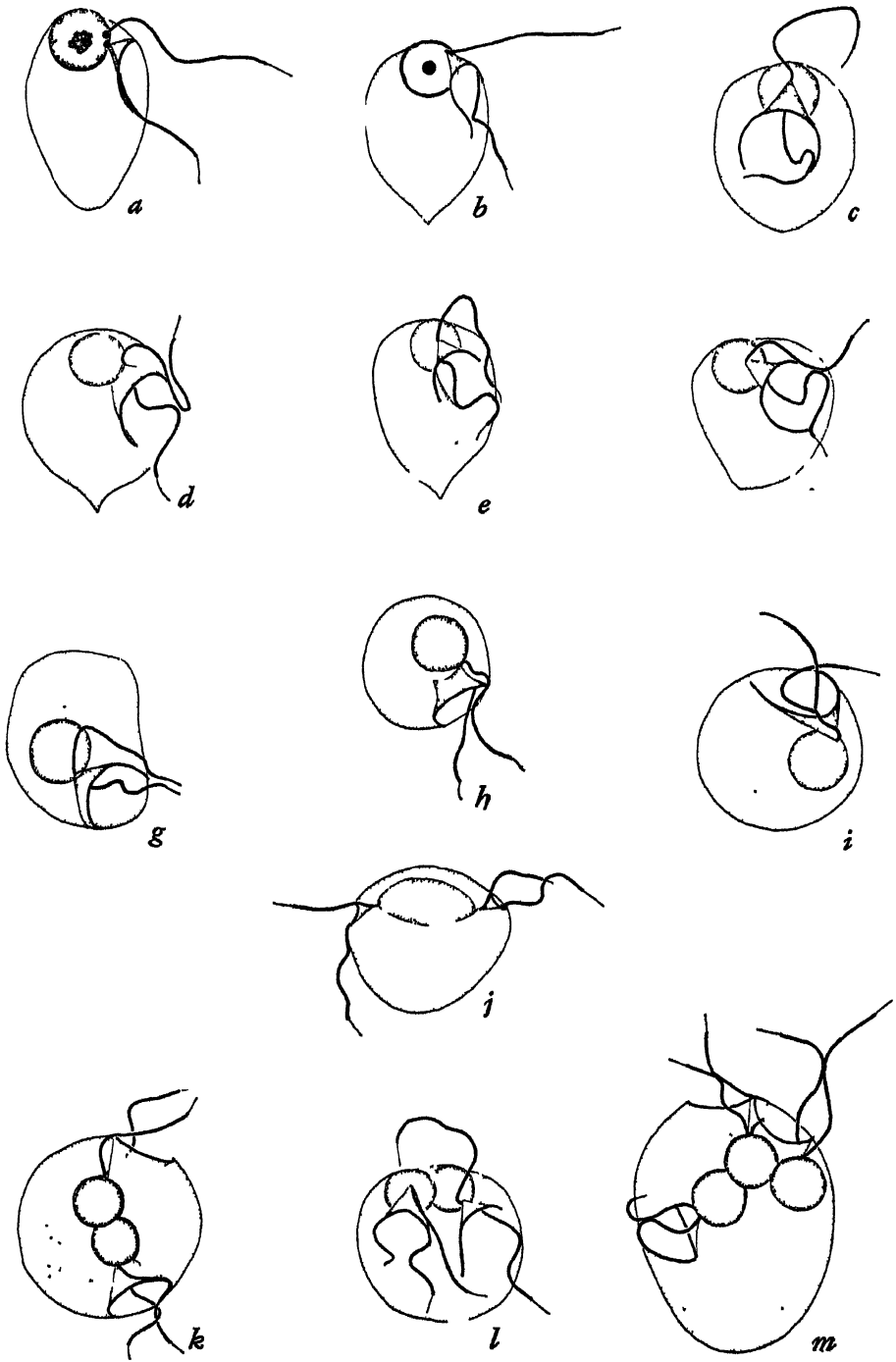
d-i. Figures of the cytostome, with the nucleus and the two flagella, showing the continuous curved marginal fibril and the triangular or trapezoidal membrane or differentiated cytoplasm that borders the depression anteriorly. In e the line within the cytostomal depression on the right side probably represents the inner margin of the wall of the cytostome.

j. Division figure, with two flagella at each pole, one a new outgrowth, and small apparently neoformed cytostomal areas, from which each of the recurrent flagella emerges.

k. Apical view of double individual, both mastigonts fully developed.

l. Ventral view of double individual, each mastigont fully developed, division of cytosome evidently delayed. $4.3\mu \times 4.7\mu$.

m. Triple individual, with three fully developed sets of flagella and cytostomal structures. Longest diameter 7μ .



THE PODOPTERA EFFECT IN DROSOPHILA MELANOGASTER

BY

RICHARD B. GOLDSCHMIDT, ALOHA HANNAH,
AND LEONIE KELLEN PITERNICK

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THE PODOPTERA EFFECT IN *DROSOPHILA* *MELANOGASTER*

BY

RICHARD B. GOLDSCHMIDT, ALOHA HANNAH,
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INTRODUCTION

IN *Drosophila melanogaster*, within the past fourteen years hereditary conditions were found which had in common an effect upon the wings, of the type classed as "homoeotic changes, substitution of serial (segmental) homologues for each other or for part thereof." The first two cases had been described earlier as new homoeotic mutants, namely, tetraltera (abbr.: tet), or transformation of wings into halteres (Goldschmidt, 1939), and podoptera (abbr.: pod), transformation of wings into leglike structures (Goldschmidt, 1945). Tetraltera was made the subject of a thesis by Villee (1942), in which both morphology and genetics were presented; but for podoptera thus far only its interesting morphology has been described (Goldschmidt, 1945). When our work on podoptera was practically finished, it became known to us that Rapoport (1942) had published a paper in Russia describing what is clearly another podoptera type. Because thus far he has presented only an account of the morphology, his paper will be reviewed along with the discussion of the morphology of podoptera. Since the discovery of tetraltera and podoptera (in 1934) a number of similar forms were isolated by Aloha Hannah and Leonie Kellen Piternick and studied in detail. Although each one of the group—and many others observed but not studied—has its specific features, all exhibit the transformation of wings into leglike structures, including all intermediate stages, and are therefore also termed podoptera. The genetic analysis of all these forms revealed that their description in terms of individual mutants, such as eye-color mutants, would not do justice to the case. It is more probable that these different variants of the general podoptera effect are not based upon different single mutant loci but that each is controlled by a group of loci, different in each case but nevertheless always resulting in the podoptera phenotype. We speak, therefore, at least for the time being, of a *podoptera effect*, thus stressing the similarities in phenotypic action rather than the genetic similarities. The reason for including the type tetraltera in the group in spite of its rather specific peculiarities will be presented below.

The work on the entire group was done by the three authors in close collaboration, and the division of labor was such that each experimented predominantly with the types he had found. Thus, the work on tetraltera and podoptera G was done by Goldschmidt, that on podoptera H by Hannah, and that on podoptera K and podoptera M(3)124 by Piternick. In addition, most of the morphological data included in this paper are based on a special study by Hannah; much of the work on Minutes was done by Piternick; and the work on dominants and their interrelation with Minutes and podoptera

was done by the senior author. The studies of salivary chromosomes were made by Hannah, and in some of the allelism tests and replacement of chromosomes all authors collaborated. Instead of presenting the work of each author separately we thought it preferable to present the whole as a monograph; but the senior author wants to emphasize that this does not mean to imply a deduction from the junior authors' share of independent work.

PART I. OCCURRENCE; MATERIAL; MORPHOLOGY

1. MATERIAL

A. UBIQUITY OF THE PODOPTERA EFFECT

After we had become well acquainted with the diverse morphological features of the podoptera effect, we came to the conclusion that podoptera types are not at all rare but are practically ubiquitous in *Drosophila* stocks. One of the reasons that their hereditary nature was overlooked in all the previous years of *Drosophila* work is that their penetrance is normally very low, particularly in mass breeding. Goldschmidt found a good specimen in 1934 and outcrossed it, but since he did not find another one in a large F_2 he assumed that it was a nonhereditary modification. Not until many years later, when he found a specimen with a well-developed wingleg, was he led to isolate a line with sufficiently high penetrance for further study. As we have since isolated lines with different percentages of penetrance ranging from 0.25 to 100 per cent, it might safely be assumed that lines exist with so low a penetrance that in an ordinary outcrossing test they would pass as normal. We could show that in each case in which we took the trouble to investigate the heredity of podoptera, even in lines with low penetrance (below 1 per cent), a line could be isolated which bred true for the production of podoptera in a certain percentage. Therefore, it may safely be assumed that in all the stocks in which podoptera individuals were found regularly, the hereditary podoptera effect was present.

In order to show the ubiquity of podoptera flies in different lines, some of the data have been tabulated (table 1); many more cases have been seen of which the details have not been recorded.

a. Podoptera G in a series of inbreeding experiments

The experiments were originally made for a study of mutation (Goldschmidt, 1947), and podoptera was found incidentally and studied. The incidence of podoptera in the inbred lines is tabulated in table 1. The interpretation of this table requires further comment: (1) The lines 8–12 were originally derived by mutation from the stock plexus blistered (abbr.: px bl.)—line 2 in table 1—as described in detail in Goldschmidt *et al.* (1945a); they were kept as separate stocks for many years prior to this experiment. Line 4 was kept heterozygous for the two marked first chromosomes, and by continuous backcrossing to the stock $In(1)y^{px\ bl}$, $In(1)w$ the autosomes became more and more those of that stock (i.e., line 8). Lines 6 and 7 show features parallel to line 4. Line 5 is the reciprocal of line 4 so far as backcrosses were made to the Bld stock into which part of the X chromosome from line 7 was inserted. (2) The Canton stock (no. 1) was the standard stock, and it produced only a few podoptera flies. In later mutation experiments an isogenic Canton stock was used as the wild-type stock. It contained so high a percentage of pod flies that it had to be discarded from work with podoptera. A line yielding a high percentage of podoptera was isolated from Canton stock but not used for further work. (3) The individual lines showed the details set forth on the following pages.

a) In line 2 (derived from the ancestral stock which had been bred for about twelve years) one podoptera fly appeared in one brood of the first generation of the mutation experiment. It should be stated here that in the following description a brood or bottle always means the offspring of a one-pair mating, except when stated otherwise. The succeeding generations were *not* derived from this brood, and were always bred from normal flies. Up to F_{14} , 0.06 per cent pod flies were found in 4 (= 8.5 per cent) of the bottles,

TABLE 1

NUMBER OF PODOPTERA FLIES IN A SERIES OF INBRED LINES BRED UNDER IDENTICAL CONDITIONS IN SINGLE-PAIR BROTHER-SISTER MATINGS
(One bottle = one single-pair mating)

Line	No. of gen.	No. of bottles	No. of flies	Bottles with pod	No. of pod flies	Percentage of bottles with pod	Percentage of pod
1. Canton.....	25	100	20,513	3	3	3.0	0.015
2. plexus blistered.....	22	123	16,701	23	16	10.0	0.14
3. plexus blistered isogenic....	17	97	14,929	13	15	13.0	0.10
4. T(1;2)Bld/In(1) $y^{px} bl$, In(1)w.....	24	172	25,707	96	267	56.4	1.00
5. T(1;2)Bld,In(1)w/In(1) $y^{px} bl$,In(1)w $\times$ T(1;2) Bld,In(1)w.....	14	53	4,041	30	59	56.6	1.40
(phenotypes mated Bld w $\times$ Bld w)							
6. CLB/In(1) $y^{px} bl$,In(1)w....	13	62	9,523	11	15	18.0	0.20
7. T(1;2)Bld/In(1) $y^{px} bl$	25	91	9,871	7	13	7.7	0.13
8. In(1) $y^{px} bl$,In(1)w isogenic..	18	116	20,347	26	32	22.0	0.16
9. In(1) $y^{px} bl$,In(1)w not iso- genic.....	23	93	15,620	14	22	15.1	0.14
10. svr^{pod}	26	290	29,134	15	21	5.2	0.07
11. svr^{pod} isogenic.....	12	65	8,517	10	20	15.1	0.24
12. In(1) $y^{px} bl$	23	86	13,097	9	9	10.5	0.07
Σ	..	1,348	188,000	257	492	19.0	0.26

none of them derived from broods with pod flies in the ancestral broods. In F_{15} , 5 pod flies suddenly appeared in a brood of 117 flies (4.2 per cent). The succeeding generations (16–22) were not bred from this but from cousins, i.e., broods without pod. In each generation, except F_{18} , pod flies appeared, namely, 0.21 per cent pod (in all flies) found in 16.7 per cent of the bottles. This is interpreted as meaning that in F_{15} one or more of the existing podoptera factors had become homozygous, rarely all of them (the bottle with 4.2 per cent). Extraction of good podoptera lines from lines of similar behavior agrees with this interpretation. But the fact that in the fourteenth generation a more potent pod factor had appeared by mutation cannot be disregarded. The isogenic line (no. 3) shows a different behavior. The percentage of podoptera is small throughout, and the occurrence is so scattered, with many generations without pod, that one must assume that in making the stock isogenic only one of the pod components with low penetrance was selected.

b) Line 4 (crossing T(1;2)Bld with In(1) $y^{px\ bl}$, In(1)w of the same derivation as line 8) has the relatively high incidence of 1 per cent pod among all flies. Here, selection for pod had actually been exercised. In F₄ the first individual with a real wingleg was found, and all further generations were derived from this bottle. (The stock pod-G, analyzed in these pages, is derived from this line.) The following generations show:

F ₄ -F ₁₁ 69 bottles	29 with pod = 42 per cent
all bottles	12,059 flies with 66 pod = 0.5 per cent
pod bottles	7,135 flies with 66 pod = 0.8 per cent
F ₁₂ -F ₂₄ 95 bottles	66 with pod = 70 per cent
all bottles	13,153 flies with 200 pod = 1.5 per cent
F ₂₀ -F ₂₄ 38 bottles	31 with pod = 82 per cent
all bottles	5,623 flies with 105 pod = 1.8 per cent

We conclude from these facts that, near F₁₂, homozygous pod factors had been selected, and probably had again been selected near F₂₀.

c) Line 5 (crossing Blond males into which the white-inversion had been inserted by crossing over with females heterozygous for the same X chromosome and In(1) $y^{px\ bl}$, In(1)w from no. 8) had the highest average percentage of pod flies. The details are: In F₁-F₉ 44.8 per cent of the bottles had pod, with an incidence of 0.9 per cent. In F₁₀-F₁₄ 71 per cent of the bottles contained pod, with 2.1 per cent total incidence. But in this case it is more probable that environmental factors were responsible. F₁₂ and F₁₃ were bred during a heat wave when the temperature in our incubators rose above 25°. Heat usually does not increase the penetrance of pod, but, in this particular case of temperature variation, up to lethal temperature the pod flies had a better rate of survival, so that actually 80 per cent of the broods had 3.4 per cent pod. In this case also, an experiment was made which showed the presence of homozygous pod in the line. Cold enhances the penetrance of pod (see below). F₆ was raised at 16°, and 100 per cent of the bottles contained pod, namely 4.9 per cent. The following data illustrate the correctness of this interpretation:

1. All generations except F₆, F₁₂, F₁₃ = 0.9 per cent pod
2. F₆, F₁₂, F₁₃ = 4.0 per cent pod (F₆ = 4.9 per cent; F₁₂, F₁₃ = 3.4 per cent)
3. F₁₀, F₁₁, F₁₄ = 1.5 per cent pod
4. F₁-F₅ = 0.7 per cent pod

d) Line 6 (CIB/In(1) $y^{px\ bl}$, In(1)w) shows again that certain pod factors, present in the line, were made homozygous by inbreeding: In F₁-F₉ only 14 per cent of the broods had 0.09 per cent pod. In F₁₀-F₁₃ 43 per cent of the broods had 0.4 per cent pod.

e) Line 7 (T(1;2)Bld/In(1) $y^{px\ bl}$), is a case in which, by chance, selection led to more homozygosity. In the first 11 generations only 2 pod flies were found in 2 bottles out of 40 (3,777 flies) = 0.05 per cent pod. The succeeding generations all descended from the F₁₁ brood with pod, and among them 5 more generations came from bottles containing pod. F₁₂-F₂₄ contained 10.6 per cent of the bottles with 0.16 per cent pod—a threefold increase.

f) Remarkable is the selection effect visible in the isogenic line 8. (The mutant line containing the y and w inversions made isogenic.)

F ₁ -F ₄	29 bottles	3,858 flies, no pod
F ₅ -F ₁₀	72 bottles	10,099 flies, 16.7 per cent broods have 0.15 per cent pod (16)
F ₁₁ -F ₁₈	45 bottles	6,390 flies, 33.2 per cent broods have 0.25 per cent pod (16)

No phenotypically pod flies were selected for breeding. In a line that is truly isogenic, no selection should be possible. But it must not be forgotten that in the isogenic stocks made by Muller's standard procedure (DIS 6), the fourth chromosome is neglected and the third chromosome is not sufficiently protected from crossing over. Thus, we may assume that an originally minute penetrance based upon second- and first-chromosome factors was increased by selection of third- and fourth-chromosome factors. But the possibility of mutation to a pod factor must also be kept in mind (see below: selection).

g) No. 9 is the line from which the isogenic line, just discussed, was derived. Here, again, no pod flies were present in the first 5 generations with 18 bottles and 3,715 flies.

In F₅-F₁₀ podoptera flies were very rare, i.e., 7.7 per cent of the bottles contained pod flies; these were 0.05 per cent pod among 8,260 flies in all bottles.

In F₂₀ a sudden increase occurred, as follows:

F ₂₀	5 bottles	1,327 flies	6 pod in 3 bottles (0.45 per cent in all bottles)
F ₂₁	7 bottles	1,236 flies	5 pod in 4 bottles (0.40 per cent in all bottles)
F ₂₂	3 bottles	420 flies	0 pod in 0 bottles
F ₂₃	4 bottles	662 flies	7 pod in 3 bottles (1.1 per cent in all bottles)

I.e., 52.6 per cent of the bottles had 0.5 per cent pod—a tenfold increase. This, again, looks like homozygous selection of a number of factors of individually minute effect, or of one especially potent one.

h) Line 10 behaved differently (the mutant *svr^{pod}* was originally derived from px bl). Here, the first 15 generations with 116 bottles and 16,819 flies did not contain any pod flies. Thenceforth, each generation beginning with 2 broods derived from the same F₁₄ grandparents, and continued by inbreeding exclusively, contained pod flies (except F₂₂ with 999 normals). The percentage remained low in F₁₆-F₂₂ descendants of these two broods, as follows:

F ₁₆	91 bottles	1,488 flies	2 bottles with pod	2 pod flies in all bottles
F ₁₇	12 bottles	1,779 flies	1 bottle with pod	1 pod fly in all bottles
F ₁₈	10 bottles	1,193 flies	1 bottle with pod	1 pod fly in all bottles
F ₁₉	9 bottles	1,203 flies	1 bottle with pod	1 pod fly in all bottles
F ₂₀	13 bottles	1,526 flies	1 bottle with pod	1 pod fly in all bottles
F ₂₁	5 bottles	661 flies	1 bottle with pod	2 pod flies in all bottles
F ₂₂	7 bottles	999 flies	0 bottles with pod	0 pod flies in all bottles

(i.e., 5 per cent bottles and 0.09 per cent pod flies.)

In F₂₃ a sudden increase took place:

F ₂₃	5 bottles	416 flies	1 bottle with pod	1 pod fly in all bottles
F ₂₄	7 bottles	1,255 flies	4 bottles with pod	7 pod flies in all bottles
F ₂₅	12 bottles	1,472 flies	2 bottles with pod	4 pod flies in all bottles
F ₂₆	3 bottles	323 flies	1 bottle with pod	1 pod fly in all bottles

(i.e., 29 per cent bottles and 0.37 per cent pod flies.)

Either pod appeared by mutation in the fourteenth generation, or pod factors were present but were kept heterozygous by unknown balancers from which they were freed in F_{16} . The genetic composition of the svr^{poi} stock as given in Goldschmidt *et al.*, 1945a, pp. 311 ff., is in favor of the latter assumption, as the stock contains a number of small translocations. (Actually, we did not succeed in making svr^{poi} isogenic for the first chromosome.)

i) Line 11 is this line, isogenic except for the first chromosome. Again, there was no pod in F_1-F_5 in 32 bottles with 4,149 flies.

F_6-F_9	21 bottles	3,020 flies	2 pod in 2 bottles
	(i.e., 10 per cent bottles and 0.07 per cent pod)		
$F_{10}-F_{12}$	12 bottles	1,348 flies	18 pod in 8 bottles
	(i.e., 66.7 per cent bottles and 1.5 per cent pod)		

The alternatives are again the same as under point *g*, etc., selection or mutation.

k) Line 12, too, is interesting (it is the homozygous $In(1)y^{pxbl}$). There were no pod flies in F_1-F_9 , with 31 bottles and 3,789 flies. Seven generations scattered over $F_{10}-F_{23}$, with 31 bottles, 5,423 flies, did not contain pod flies. The other 6 generations, with 24 bottles, 3,885 flies, had 9 bottles containing 9 pod. That is, in $F_{10}-F_{23}$, 16.4 per cent bottles contained pod, and there was 0.1 per cent pod in all bottles. In the 6 generations with pod, 37.5 per cent bottles had 0.23 per cent pod. The most likely explanation is that by chance the pod factors remained heterozygous, except in a few bottles with some homozygosity.

b. Podoptera in the Florida stocks

Podoptera flies were first observed in the Fla Iso '39 strain¹ in 1943, both in the stock bottles and among the flies in an experiment involving crosses with ClB. Because of a relatively high frequency of podoptera in this Florida strain, a comparative study was made with the other Florida strains (Carnegie Fla '19, Columbia Fla, Amherst Fla '40, and Amherst Iso '44). All the strains are closely related; two of them (Iso '44 and Iso '39) are isogenic, and one of these (Iso '44) was derived from Florida 40.

Inbred lines were maintained by single-pair matings of phenotypically normal flies for 20 generations. Because reduced viability and fertility as well as the accumulation of lethal or detrimental factors increased with inbreeding, 2 lines were carried simultaneously for each strain. Four matings were made from each line in each generation, but the offspring of only one were counted and used as parents for the next generation. The results of inbreeding each strain for 20 generations are summarized in table 2.

¹ The original stock, Fla 10, was probably that of Morgan at Columbia (Bridges and Brehme, 1944). It was made isogenic in 1936 by Child and again by Ives in 1939. Ives found, in the process of making Fla Isogenic stock '39, that "the old stock had a high frequency of one or more third-chromosome lethals, but that it gave wild-appearing flies, however, frequently with crumpled legs (personal communication from P. T. Ives to R. Blanc, Dec. 29, 1939). Bridges (Bridges and Brehme, 1944) reported that the old stock contained the inversion (3R)Payne, and Kodani (Goldschmidt, Gardner, and Kodani, 1939) found that most of the flies of the Iso '39 stock had the same inversion.

Columbia, Fla '19 and Fla '40 lines gave similar incidences of podoptera, i.e., 0.13, 0.14, and 0.13 per cent, respectively. Likewise, the podoptera showed no clumping in specific generations (table 3), a fact which indicates that although the penetrance is low the incidence is relatively stable. The incidence in the Iso '44 lines had greater variance. These data are significant in two respects. The stock was made isogenic from the Amherst line by Ives (Bridges and Brehme, 1944). With the exception of the fourth chromosome and the

TABLE 2

INCIDENCE OF PODOPTERA IN FIVE FLORIDA STRAINS, BRED UNDER IDENTICAL CONDITIONS AND BY BROTHER-SISTER MATINGS FOR TWENTY GENERATIONS

Stock	Gen.	No. of bottles	No. of flies	Bottles with pod	pod	Per-centage bottles with pod	Per-centage of pod	Total flies	Total pod	Per-centage of pod
Col Fla										
A.....	20	20	1,607	2	3	10.00	0.18			
B.....	20	20	1,566	1	1	5.00	0.06	3,173	4	0.13
Fla '19										
A.....	20	20	1,420	2	2	10.00	0.14			
B.....	20	20	1,431	2	2	10.00	0.14	2,851	4	0.14
Fla '40										
A.....	20	20	2,621	3	3	15.00	0.11			
B.....	20	20	2,914	3	4	15.00	0.14	5,535	7	0.13
Fla Iso '44										
A.....	20	20	3,426	3	10	15.00	0.29			
B.....	20	20	3,232	4	6	20.00	0.19	6,658	16	0.24
Fla Iso '39										
A.....	20	20	1,543	13	29	65.00	1.87			
B.....	20	20	1,332	8	13	40.00	0.98	2,875	42	1.46

regions of the other chromosomes that may have undergone crossing over in the process of making the stock isogenic, all the loci were homozygous; therefore the podoptera factor or factors also should have become homozygous. Although the incidence of podoptera is greater in the isogenic line (Iso '44) the difference between it and the nonisogenic line (Fla '40) is not statistically significant ($\chi^2 = 1.97$). The apparent increase in incidence of podoptera in line A of Iso '44 in the sixth to tenth generations, and again in the sixteenth to twentieth generations, might be accounted for by chance segregation of new podoptera factors which had arisen since the line was made isogenic.

All the lines were kept by nonselected mass matings after the twentieth generation. When a low-incidence podoptera strain was needed in 1946 for further analysis, the two lines Columbia (B) and Fla '40 (A) were again tested for incidence of podoptera. Four single-pair matings were made with phenotypically normal flies from each line. In the F_1 and succeeding generations,

all the flies were recorded, but the parents for the four F_2 and F_3 matings were phenotypically normal flies selected from one bottle. The data are summarized in table 4. The incidence of podoptera had not changed significantly ($\chi^2 = 1.06$) in the Columbia line, but the Amherst Fla '40 line showed an increase in penetrance, and a significant difference ($\chi^2 = 33.3$). Because of the low incidence of podoptera (0.06–0.39 per cent), the Columbia line was used as the low podoptera stock in the later experiments.

TABLE 3
INCIDENCE OF PODOPTERA BY GENERATIONS IN THE INBRED FLORIDA LINES

Stock	Generations 1-5		Generations 6-10		Generations 11-15		Generations 16-20		Total pod
	Bottles with pod	No. pod	Bottles	No. pod	Bottles	No. pod	Bottles	No. pod	
Col Fla									
A.....	..	..	1	2	..	..	1	1	3
B.....	..	..	..	..	1	1	..	..	1
Fla '19									
A.....	..	..	1	1	..	..	1	1	2
B.....	..	..	1	1	..	..	1	1	2
Fla '40									
A.....	1	1	..	..	1	1	1	1	3
B.....	1	1	1	2	..	..	1	1	4
Fla Iso '44									
A.....	..	..	1	3	..	..	2	7	10
B.....	..	..	3	3	1	3	..	..	6
Fla Iso '39									
A.....	3	4	3	8	3	9	4	8	29
B.....	3	4	1	3	2	2	2	4	13

The Fla Iso '39 inbred lines gave podoptera offspring in almost every generation. Line B showed no peculiarities in incidence, having one or two podoptera per bottle as would be expected in random sampling. Line A, however, had an increase of podoptera in the eighth generation (5 podoptera among 121 flies, or 4.13 per cent. See table 5). From the ninth to the twentieth generations the incidence remained relatively high, although with considerable fluctuation. These data suggest the possibility that either segregating podoptera factors had become homozygous or that a new factor had arisen by mutation.

The fluctuations in incidence of podoptera flies in the first five generations of the Fla Inbred line suggested that the podoptera factor or factors may have been heterozygous and that chance segregation would have given the results obtained. A number of the phenotypically low-grade pod flies from the first generation were also mated. The F_1 offspring included no typical podoptera phenotypes. A mass mating gave no podoptera in the F_2 generation, but the

TABLE 4
INCIDENCE OF PODOPTERA FLIES IN TWO STRAINS OF FLORIDA

Generation	Col. Fla.						Fla. 40			
	Bottles			Flies			Bottles		Flies	
	Total no.		With pod	Total no.	pod	Percentage	Total no.	With pod	Total no.	Percentage
1.....	4	..	..	431	..		3	2	414	0.48
2.....	3	1	1	162	2	1.23	4	2	336	1.19
3.....	4	1	1	181	1	0.55	4	4	370	2.16
Σ.....	11	2	2	774	3	0.39	11	8	1,120	1.25

TABLE 5
INCIDENCE OF PODOPTERA PER GENERATION IN LINE A OF INBRED FLA ISO '39 STOCK

Generations 1-5					Generations 6-10					Generations 11-15					Generations 16-20				
Generation	No. of flies	pod	Percentage		Generation	No. of flies	pod	Percentage		Generation	Flies	pod	Percentage		Generation	Flies	pod	Percentage	
F ₁	41	..			F ₆	70	..			F ₁₁	11	..			F ₁₆	115	2	1.74	
F ₂	53	1	1.88		F ₇	29	..			F ₁₂	65	2	3.08		F ₁₇	104	2	1.92	
F ₃	88	2	2.27		F ₈	121	5	4.13		F ₁₃	94	4	4.26		F ₁₈	146	2	1.37	
F ₄	42	..			F ₉	92	2	2.17		F ₁₄	102	3	2.94		F ₁₉	68	..		
F ₅	46	1	2.17		F ₁₀	43	1	2.28		F ₁₅	144	..			F ₂₀	69	2	2.90	
Total	270	4	1.48			355	8	2.25			416	9	2.16			502	8	1.59	

progeny of six matings of phenotypically normal flies included (in F_3) phenotypes of blistered wing, vestigial wing, low-grade podoptera types, spread wing, etched abdomen and one first-chromosomal mutant, probably an allele of *tan*. The F_4 progeny included a typical podoptera type V male, and a gynandromorph. Essentially the same phenotypes appeared in the F_1 - F_3 generations on inbreeding any one of the abnormal phenotypes, i.e., etched abdomen, gynandromorph, podoptera, found in the inbred lines.

When no podoptera appeared in the sixth and seventh (inbred) generations, four matings were made from the phenotypically normal F_6 and F_7 flies; and, as before, various phenotypes were selected as parents for the succeeding generations. In spite of the fact that neither the F_6 nor the F_7 generation had

TABLE 6
PENETRANCE OF PODOPTERA FROM THE SIXTH AND SEVENTH GENERATION FLIES
IN THE INBRED FLORIDA LINE

Generation	F_6 line			F_7 line		
	No. of flies	pod	Percentage	No. of flies	pod	Percentage
F_6	70	..		...	..	
F_7	449	16	3.56	29	..	
F_8	239	3	1.25	490	20	4.08
F_9	...	..		219	6	2.74
F_{10}	...	..	...	426	19	4.46
F_{11}	...	..		628	30	4.78

podoptera, their progeny gave the highest incidence yet found for the Fla strain (table 6). Furthermore, this high penetrance was retained in subsequent generations. Therefore it seems likely that, sometime preceding the sixth or seventh generation, either segregating podoptera factors became homozygous or a new podoptera mutant appeared and was selected by chance.

Because the Fla Iso '39 strain had a high incidence of podoptera it was chosen by Hannah for further analysis of podoptera and is therefore designated pod-H.

c. Podoptera in other wild-type stocks

It has already been stated that wild-type stocks other than Florida were found to contain podoptera (e.g., Canton, table 1). The Oregon stock also contains pod factors of low action; therefore an occasional pod phenotype is found in Oregon stocks.

d. Podoptera in dominant marker stocks

We shall report below, when discussing localization experiments, the presence of pod in standard marker stocks. Pod flies were also found in the Bd/In(3R)C stock and, with a rather high incidence, in a standard Bx stock. None of these stocks has been used for work on podoptera itself, but we shall discuss in detail the special features of Bd and Bx which make them important for the solution of the podoptera problem.

e. Podoptera in Minute stocks

Podoptera flies are very frequent in Minute stocks. The details of this relation will be presented below. In table 8 some data are presented which demonstrate the practically ubiquitous presence of pod in Minute stocks. In 1944 some of the third-chromosome Minutes were checked for the occurrence of podoptera. All of them contained pod in the stock bottles: M(3)124 many of high grade, M(3)B² many of low grade, M(3)S34 few high grade, M(3)w few high grade, M(3)36e the same.

TABLE 7
PODOPTERA IN MINUTE STOCKS

Stock	No. of flies	Percentage of pod	Expressivity
M(3)124/In(3R)C,e,1(3)e..... (a)	195	7.1	Class VI mostly
..... (b)	709	1.5	Class VI mostly
M(3)B ² /In(3R)C,Sb,e,1(3)e.....	864	6.9	Class I only
M(3)w/In(3R)C,e,1(3)a.....	860	0.8	
M(3)36e/In(3R)C,e,1(3)a.....	1,252	1.5	Class I only
M(3)S34/T(2;3)Mé.....	763	0.2	
M(2)S5/Pm ds ^{33k}	317	...	
M(2)S7/Cy al ² lt ³ L ⁴ sp ²	748	5.0	Class I only
M(2)S11/Pm ds ^{33k}	345	0.3	Class I only
M(2)S12/Pm ds ^{33k}	47	2.0	Class I only
M(2)S13/Pm ds ^{33k}	643	0.3	Class I only
M(2)S13/Cy,bw ³⁴	844	2.1	Classes I and III
M(2)173/Cy al ² lt ³ L ⁴ sp ²	746	0.4	Class I only
M(2)l ² /Cy L ⁴ sp ²	368	0.3	Class I only
M(1)n/In(1)AM.....	759	0.3	Classes II, III, VI
M(1)Sp/In(1)AM.....	468	3.4	Classes I and II

At some later time the offspring of a series of single-pair matings from these stocks was checked for the penetrance of podoptera and for the expressivity of the podoptera phenotype. A summary of the results is presented in table 7. The table includes also data on several second- and two first-chromosome Minute stocks. The penetrance of podoptera was less than 1 per cent in most of the stocks, but there were several stocks with a higher incidence of podoptera. In the stocks M(3)124 and M(3)B² the penetrance of podoptera was relatively high (7.1 per cent and 6.9 per cent, respectively). In all the stocks, pod flies of class I were far more common than any other type, with the exception of the M(3)124 stock, which contained all podoptera types with an especially high frequency of class VI.

f. Podoptera in Drosophila literature

The *Drosophila* literature contains so many descriptions or mention of what clearly was podoptera—including mutants that were not further studied—that it is hardly possible to collect all instances. We mention only a picture of a typical podoptera given by Ma (1943), who believed that he had produced it

by temperature action. The only author who actually found podoptera and realized its importance is Rapoport (1942), as already mentioned in the introduction. He had the good luck to find a dominant line with almost 100 per cent penetrance and high expressivity. (He called this Met and considers it a simple second-chromosome mutant.)

B. THE LINES CHOSEN FOR STUDY

For the present study, five major lines (plus some sublines) were chosen because of their relatively high penetrance.

a. *Tetraltera*

As indicated above, we consider now the type tetraltera (abbr.: tet), originally described as a simple mutant, as a part of the podoptera group, although the phenotype separates it partly from the other podoptera types. The reasons are: (a) tetraltera (see below: morphology) frequently produces in its extreme form a regular wingleg instead of a haltere-like structure. The stages leading up to the wingleg are different, it is true, from those of podoptera in that in pod the wingleg develops only from the costal cell of the wing, whereas in tet the whole wing is reduced until all of it has been narrowed down to the extent of the costal cell. The last but one stage is the haltere-like structure. Thus, the tet factors must affect different embryological processes, but the end result may be identical with that of pod. Compare, in fig. 1, *e* (tet) with *d* (pod); see discussion below. (b) The genetics of tet, which originally was considered a simple third-chromosome mutant, turned out to be very similar to that of pod, including multiple-factor inheritance and also pleiotropic effects.

The origin of tet in one of the senior author's earlier experiments has been reported (Vilee, 1942, p. 126). The offspring of the first isolated line with a rather low penetrance (± 5 per cent) has been bred for about fourteen years. Vilee, for his work, isolated a series of sublines by selection or by isolating other mutants which had appeared. When the progress of the podoptera work necessitated a restudy of tetraltera only three stocks were left: one with penetrance so low that it was unsuitable for analysis; one with good penetrance after a time of selection; and a similar one in which a mutant with blistered wings had become homozygous. In the course of the work an enhancer derived from a Minute stock was introduced into the tetraltera stock and thus produced a useful stock with practically 100 per cent penetrance of tet, called tet-100.

b. *Podoptera G*

The line was derived from the pod individuals obtained in the cross $T(1;2)Bld \times In(1)y^{px\ b1}, In(1)w$ always backcrossed to the latter (see table 1, line 4). In the eighth generation it was possible to obtain offspring from a pair of podoptera phenotypes, both homozygous for the two rearrangements in the *y* and *w* regions (thus, pod *G* is marked by these two position effects). (Henceforth a "pod-individual" refers to a phenotypically pod fly, and a mating pod $\times +$ means, even within a pod line, the respective phenotypes.) The offspring contained 3 pod in 272 flies = 1.1 per cent pod. F_2 by pair mating produced 475

normal flies and 3 pod = 0.64 per cent pod. F_2 in mass culture gave 3,210 flies and 5 pod = 0.16 per cent pod. A second mating pod $\times$ pod was obtained in the ninth generation of line 4. The result was 298 normal flies and 1 pod; a third similar mating gave 1 pod in 173 flies, i.e., rather low penetrance. Another try in F_{10} (i.e., pod $\times$ pod) of the same line 4 gave better penetrance, namely, 148 flies and 2 pod = 1.3 per cent; an F_2 from normal parents (4 matings) produced 1,130 flies and 7 pod = 0.6 per cent. In the same generation an unrelated mating of normal flies from normal parents produced the unusually high ratio of 5 pod in 161 flies = 3.1 per cent. The data from two generations of offspring (pair mating) from this brood are summarized in table 8. From this

TABLE 8
ORIGIN OF pod-G

Generation	Cross	Total flies	pod	Percentage of pod
F_1	pod $\times$ +	200	1	0.5
F_1	+ $\times$ +	182	3	1.6
F_1	+ $\times$ +	298	7	2.3
F_2	+ $\times$ pod	207	2	1.0
F_2	+ $\times$ pod	178	2	1.1
F_2	+ $\times$ +	232	2	0.9
Σ		1,297	17	1.3

F_2 the nonisogenic lines of pod-G were derived. Simultaneously, single high-grade pod flies of different origin (but from the same line 4) were mated to the dominant marker flies y ; Cy/Pm ds^{38k} ; H/In(3R)Mo, Sb sr for production of an isogenic pod line. Two such stocks were made: one isogenic only for the second and third chromosomes, one isogenic for all but the fourth. The latter was mostly used for the analysis.

c. *Podoptera H*

This line was derived, as already stated (p. 77), from a Florida strain (Fla Iso '39) which showed a high incidence of podoptera. In five generations of inbreeding by single-pair matings of phenotypically normal flies the incidence of podoptera was 1.48 per cent. In the direct line the sixth and seventh generations had no podoptera, but a high incidence was found in succeeding generations. In the eighth generation, one bottle, 704c, gave 5.7 per cent. The incidence of podoptera among direct descendants of flies from bottle 704c was in F_2 , 2.74 per cent; F_3 , 4.46 per cent; F_4 , 4.78 per cent. This line, therefore, was chosen for analysis; it is designated pod-H.

d. *Podoptera K*

In the dominant Pointed stock (for description of stock see Goldschmidt, 1947, p. 218), podoptera flies were found occasionally, but always in very small numbers. From this stock, a male with very rough eyes and a wing phenotype

resembling that of vestigial-notched was isolated. The male was crossed to a female of the Patterson stock ($\underline{y}$; $\underline{bw}$; e^4 $\underline{ro}$; $\underline{ey}$). All the F_1 offspring looked normal. This proves that neither one of the characters is sex-linked or dominant, and that the rough-eye mutant is not an allele of rough. This character will from now on be designated as rough-like ($\underline{ro-l}$) and the wing character as notched-like ($\underline{no-l}$). In F_2 a number of $\underline{ro-l}$ $\underline{no-l}$ flies were found. One of the males was simultaneously podoptera. This male was mated to a $\underline{ro-l}$ $\underline{no-l}$ sister. After three generations of inbreeding, one culture of this line showed about 25 per cent podoptera. From this culture the stock podoptera K was established.

e. Podoptera M(3)124

Podoptera flies which appeared in the stock M(3)124 were isolated and bred, and after a few generations of mass selection a stock pod-M(3)124 with a penetrance of podoptera fluctuating around 15 per cent was established.

C. DIFFICULTIES OF GENETIC ANALYSIS

It turned out that the genetic analysis of pod met with a large number of unusual difficulties. When Villee's work on tetraltera was being done in the same laboratory, the unexpected situation arose that tet combined with the markers in all chromosomes, so that even the first step in localization proved to be a failure. This can be attributed to a sensitivity of the genetic pod action to the presence of dominants, rearrangements, and even recessive mutants, all of which may enhance dominance as well as penetrance of podoptera; or, more probably, as we shall see, to the presence of some pod alleles in all marker stocks. Naturally, the difficulties of analysis increase still more when it comes to localization within a chromosome. A second difficulty raised by the ubiquity of the pod factors is that even wild-type stocks introduce, or may introduce, podoptera factors. Therefore, in later work, only one wild-type line, Columbia Fla (p. 74)—a line which Hannah found to have the lowest incidence, almost absence, of pod flies—was used for outcrosses. A third difficulty is the apparent dominance shown by certain pod lines (see below, pp. 249-266), which again is easily influenced by genetic markers. A fourth difficulty is, of course, the low penetrance which makes localization experiments especially tedious and unreliable. Finally, there is the great irregularity of the pleiotropic effects of pod, which forced us to classify only on the basis of the wing character, except when the pleiotropic effects were studied.

2. MORPHOLOGY AND VARIABILITY

The morphology of the tetraltera and podoptera-G type has been described and discussed by Goldschmidt (1940, 1945) and by Villee (1942). For all details, reference should be made to these papers. Here we review only such facts as are relevant for the understanding of the genetic analysis. A new detailed study has been made for pod-H, because a number of special features were found which are important for the phenogenetic analysis of the phenomenon. New data on the leg effect in pod-G have also been added.

A. CLASSIFICATION AND SPECIFIC FEATURES

a. *Podoptera G*

The podoptera effect has a very variable expressivity (details and illustrations in Goldschmidt, 1945). The lowest effect consists only of a spreading at right angles of one of the otherwise normal wings—an effect which probably has been observed innumerable times by *Drosophila* workers without realizing its significance. In our experiments this is called class I.

Class II. In this there is also a wing at right angles (as in all the other classes), but the wing structure is changed. The venation is changed, especially near the posterior margin, the wing is smaller, its contours become more or less abnormal, notches may be present, also blisters, and sometimes the costal cell changes.

Class III. The wing is much shortened and is inflated to a balloon-like structure showing, however, the details of wing structure like marginal hairs and veins. There is a tendency for the costal cell and the alula to separate from the rest of the wing blade.

Class IV. The wing is still shorter and has a tendency to separate into four parts, e.g., the costal cell, the main wing blade, which is divided by a deep incision into two parts, and the alula. There is much variation in detail, hardly two wings being completely alike.

Class V. The decisive feature is the complete separation of the costal cell from the rest of the wing and its assumption of the structure of a leg without a tarsus. The posterior three sections of the wing are very variable. They may have wing or alula character, or may be transformed into irregular knobs (the details of which are figured in Goldschmidt, *op. cit.*), or may even be absent.

Class VI. Here the wingleg is reduced to an irregular palpus-like structure, or even to a little knob, and the three other parts form irregular knobs or may even be absent. In the work with pod-G no further classification was used. If both wings were affected differently, the classes were recorded for both, but the higher class was entered as the phenotype.

b. *Tetraltera*

A very similar classification was used for tetraltera. Classes I and II are generally indistinguishable from the same classes in pod, and even class III is so overlapping that it could hardly be selected from a mixture (for some details, however, see below). From class IV on, the specific differences are clear, namely, the progressive transformation of the wing as a whole into a haltere or wingleg in tetraltera, as opposed to the splitting of the wing into four sections in pod, only the first of which transforms into the wingleg (see fig. 1). Thus, class IV in tet is an intermediate condition between wing and haltere closely resembling the corresponding stage of transformation of halteres into wings in the mutant tetraptera (between *b* and *c*, fig. 1). Class V represents the typical tripartite haltere-like structure from which the name is derived (between *c* and *d*). Class VI contains all different conditions which lead beyond



Fig. 1. Semidiagrammatic representation of the differences between tetraltera (left) and podoptera (right) in a series of typical conditions.

that stage, namely, a real wingleg or reduced palpus-like structures or chitinous knobs (between *e* and *f*).

c. Podoptera H

The variability of expression of the pod-H effect was as diverse as that of pod-G, but most of the flies could be assigned to the six general classes described by Goldschmidt (1945). The flies that did not fit into these classes were rare individual variants, or phenotypes characteristic only of pod-H and likewise very rare. For the selection experiments and for purposes of comparison with podoptera in other lines, only the flies in the six classes were considered. However, this designation is purely arbitrary, for actually there is a complete transition of phenotypes from the normal wing to the wingleg.

In a morphological study the transitional types are of special significance, for from them the steps of development leading to a definite structure may be reconstructed. In the case of podoptera, which has a penetrance so low as to make an embryological study very difficult, the embryological processes may be interpreted with relative accuracy by a study of the diverse phenotypes. To facilitate such a study the six classes were further divided into subclasses, as follows:

- Class I. No subdivision.
- Class IIa. The wing is incised, giving a cut, notched or beaded-like phenotype.
- Class IIb. The wing has abnormal venation.
- Class IIc. The wing is blistered.
- Class IId. The wing blade is shorter and curved.
- Class IIe. The wing blade may be any one of the types described above; in addition, the costal cell is modified by enlargement, duplication of bristles, or has other abnormalities.
- Class IIIa. The wing is very blistered, or in extreme cases is completely inflated into a balloon.
- Class IIIb. The balloon is collapsed, or is filled with a dark fluid, or shows general degeneration.
- Class IVa. The wing is vestigial-like, but the costa shows no wingleg characteristics.
- Class IVb. The costal cell is distinct from the wing blade, which is usually further reduced; the costal cell is usually a wingleg.
- Class Va. The costal cell is duplicated and usually wingleg-like, but the wing including the normal costa is more or less normal.
- Class Vb. The wing blade is abnormal, being reduced in size, showing abnormal venation, etc.; the costal cell is separate as a wingleg.
- Class Vc. The wingleg is separated from the posterior part of the wing, which consists usually of irregular chitinized knobs; or only the wingleg is present.

Class VIa. The wingleg is reduced to a palpus-like structure, but the wing-blade tissue is still present, although it may be quite abnormal.

Class VIb. The wing blade forms irregular knobs.

Class VIc. The parapod only is present, the rest of the thorax being more or less normal.

Class VI $\bar{d}$. The wing region consists of irregular knobs with no distinct parapod.

TABLE 9
DISTRIBUTION OF PODOPTERA H FLIES IN CLASSES: INCIDENCE OF PODOPTERA
IN SUBCLASSES

Class	Single	Bilaterals								Bilaterals with higher type	Total	Σ
		With same	With I	IIa	IIb	IIc	II $\bar{d}$	IIe	Total			
I.....	39	6	..	..	..	..	..	..	45	29	74	74
IIa.....	11	..	2	..	..	..	..	..	13	5	18	
IIb.....	28	2	2	3	..	..	..	..	35	..	35	
IIc.....	29	..	1	..	..	..	..	..	30	1	31	
II $\bar{d}$	84	2	11	1	..	..	..	..	98	2	100	
IIe.....	15	..	..	..	..	..	..	..	15	2	17	201
IIIa.....	86	9	4	..	..	1	2	1	103	..	103	
IIIb.....	43	..	3	1	..	..	..	1	48	..	48	151
IVa.....	16	3	2	..	..	..	..	..	21	..	21	
IVb.....	16	..	1	..	..	..	..	..	17	..	17	38
Va.....	5	..	..	..	..	..	..	..	5	..	5	
Vb.....	2	..	1	..	..	..	..	..	3	..	3	
Vc.....	2	..	..	..	..	..	..	..	2	..	2	10
VIa.....	7	..	..	..	..	..	..	..	7	..	7	
VIb.....	11	..	..	..	..	..	..	..	11	..	11	
VIc.....	15	..	1	..	..	..	..	..	16	..	16	
VI $\bar{d}$	20	..	1	..	..	..	..	..	21	..	21	55
Total.....	429	22	29	5	..	1	2	2	490	39	529	529

In the course of the selection experiments, 490 pod-H flies were preserved for the morphological study, similar to that made by Goldschmidt for pod-G (1945). Although they were selected primarily for their characteristic phenotypes, all the podoptera types were included. Table 9 gives the distribution of the podoptera types. Four hundred and twenty-nine had only one wing affected. Of the bilaterals, 22 were symmetrical and 39 were asymmetrical. Ninety per cent of all flies were only class I, 39 per cent class II, 31 per cent class III, 8 per cent class IV, 2 per cent class V, and 11 per cent class VI. Classes II and III also had the highest incidence of bilaterals.

Of the 490 podoptera flies, 298 were females and 192 were males, or a ratio of 1.55 to 1. In the lower classes the ratio of females to males is about 1 : 1, but the higher classes had a smaller number of males (table 10). The genetic significance of this difference will be discussed in detail in later chapters.

On the basis of the classification presented above, the two events involved in the formation of the wingleg, i.e., (1) changes in the costal cell and (2) reduction of the wing blade, may be considered separately, although both processes occur simultaneously. Types I, IIa to IIc, IIIa, IIIb, and IVa are primarily changes in the wing blade, whereas classes IIe, IVb, V, and VI are more or less

TABLE 10
DISTRIBUTION OF PODOPTERA H FLIES IN CLASSES; RATIO OF FEMALES TO MALES
IN EACH CLASS

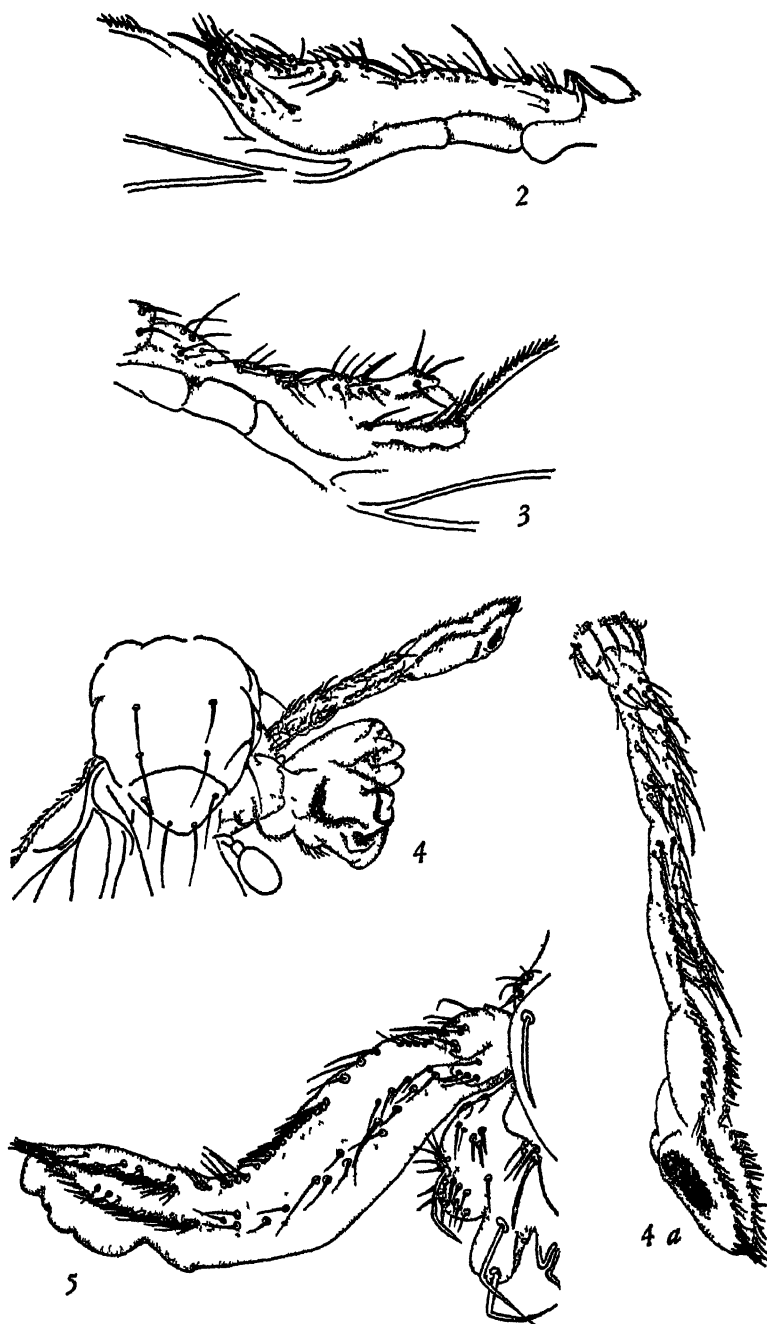
Class	Total flies		One side		Bilaterals							
	♀	♂	♀	♂	With I		With II		With III		With IV	
					♀	♂	♀	♂	♀	♂	♀	♂
I	24	21	21	18	3	3						
II	100	91	86	81	8	8	6	2				
III	109	42	96	33	2	5	4	2	7	2		
IV	22	16	20	12	2	1						3
V	6	4	6	3		1						
VI	37	18	37	16		2						
Σ	.		266	163	15	20	10	4	7	2		3

restricted to changes in the costal cell. The transitional changes from one type to another show that both the reduction of the wing blade and the formation of a wingleg may be accomplished by several series of changes which are quite distinct but may overlap.

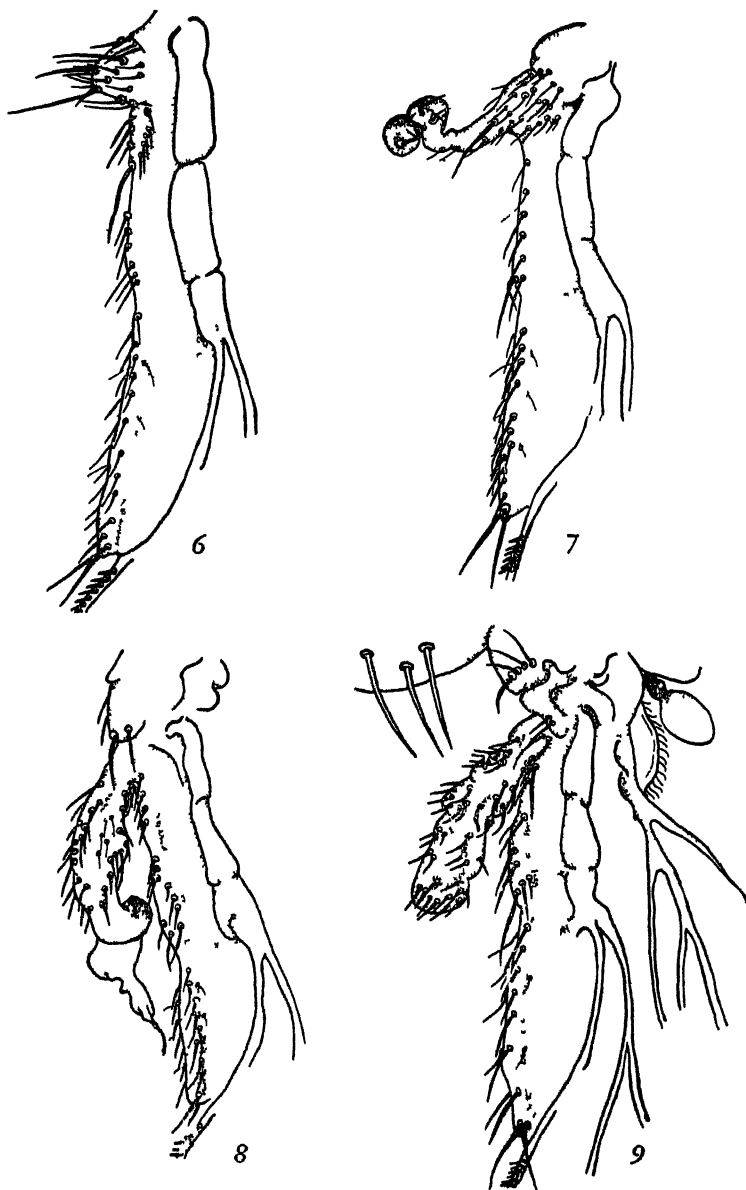
The wingleg of pod-H (and more or less that of pod-G) may be produced in four different ways; the methods by which this is accomplished differ considerably in detail but have a common basic similarity, i.e., changes in the costal cell and (or) structures adjoining the costal cell.

1. *Enlargement and duplication of the costal vein.*—The costal vein increases in size, and the rows of bristles along the margin become duplicated (pl. 12, fig. 1). Simultaneous reduction of the wing blade results in a wingleg of the type shown in fig. 24 of podoptera phenotypes described by Goldschmidt (1945). The transitional stages are given in figs. 19, 20, and 21 of the same paper. This type of wingleg is less common in pod-H than in pod-G.

2. *Mirror-image duplication of the costa, bipartite wingleg.*—The most usual method of formation of the wingleg in pod-H (not in pod-G) is by a mirror-image "duplication" of the costal vein parallel with the radial vein within the costal cell (text figs. 2, 3; pl. 12, fig. 2). The bristles on the lateral margin show few changes or duplications in the early stages but may increase in number in



Figs. 2-5. Pod-H. Fig. 2. Early stage of mirror-image duplication of the costal vein. Fig. 3. Later stage of mirror-image duplication of the costal vein. Fig. 4. Wingleg of several segments, including part of the marginal vein as well as the costa. Fig. 4a. Detail of the wingleg in fig. 4. Fig. 5. Wingleg of several segments comparable to the one in fig. 4 but having no wing blade



Figs. 6-9. Pod-H. Formation and growth of the wingleg by growth and differentiation of the humeral plate region. (Anterior edge left, posterior right.)

later stages as the thickness and size of the costa increase. The final wingleg (pl. 12, fig. 3) usually consists of two rather distinct segments.

3. *Wingleg including part of the marginal vein.*—In the next type the wingleg is longer and consists of several “segments” (text figs. 4, 5; pl. 12, fig. 9). A considerable part of the first longitudinal vein participates in the formation of this type of wingleg. The steps include separation of the marginal cell from

the wing blade, and the appearance of bristles along the edge of the marginal cell which has become separated from the rest of the wing (pl. 12, figs. 6-9). This type of wingleg differs from the first two types previously described, in that the separation from the wing probably occurs first, and the addition of bristles along the margin is a secondary feature.

4. *Wingleg produced by growth of a new structure at the base of the costal cell.*—In the first three types the processes are similar, i.e., changes in the costal cell proper. In the fourth, a quite different process occurs: the changes take place

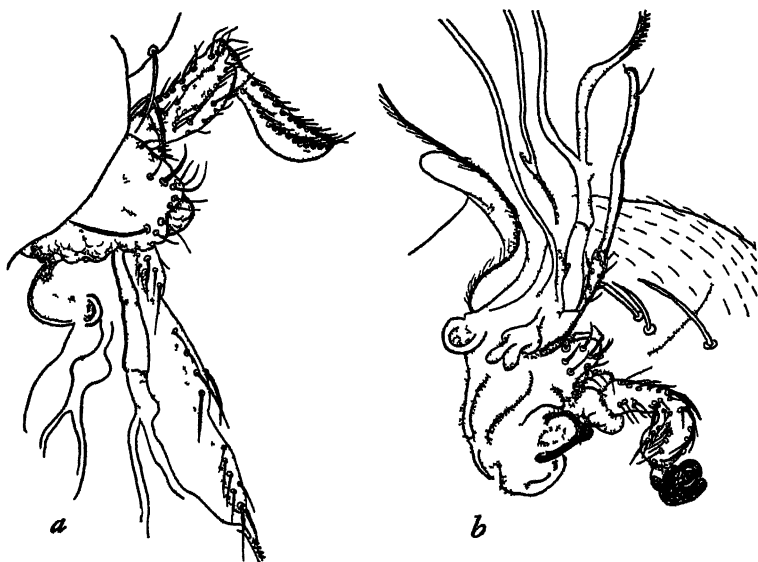


Fig. 10, a, b. Pod-H. Dorsal and lateral views of a wingleg in addition to the normal wing.

at the base of the costal cell in the humeral plate region. The first stage is an enlargement of the humeral plate (text fig. 6). As the hump increases in size, bristles appear, and with further growth the bristle pattern becomes definite and similar to that of the costal cell. In transitional stages (text figs. 7-9; pl. 12, figs. 4-5) the structure shows further enlargement until it is a fully developed wingleg with the same structure, shape, and bristle pattern as the wingleg formed by the mirror-image costal cell. In this series, however, the wing usually remains quite normal, but there may be a reduction in the size of the costal cell, and in the more extreme cases most of the bristles of the costal cell are absent. In further modification, a chitinized bulla with bristles grows between the wingleg and the wing (text fig. 10, a, b).

The reduction of the wing blade may take place in several ways also. The most frequent method is by gradual reduction of the size of the wing without loss of any specific region or change in the typical wing shape (pl. 12, figs. 11-15). Venation may be abnormal or blisters may be present, but the typical wings of this series show only gradual reduction of wing size accompanied by loss of, first, the cross veins and then the longitudinal veins in the order: L_5 , L_4 , and L_3 . Of 86 podoptera flies of this type (II d) 52 showed direct transi-

tions from the type I wing, one had plexus venation, one was blistered, and one was blistered and had a slightly abnormal costal cell. Thirteen of the type II*d* wing also had modified costal cells (II*e*). In later stages, reduction is accomplished either through degeneration, III*b* (32 cases), or inflation, III*a* (22 cases), or inflation and degeneration (5 cases). Others show further reduction of the wing blade (without other modifications), leaving only a small amount of tissue attached to the wingleg.

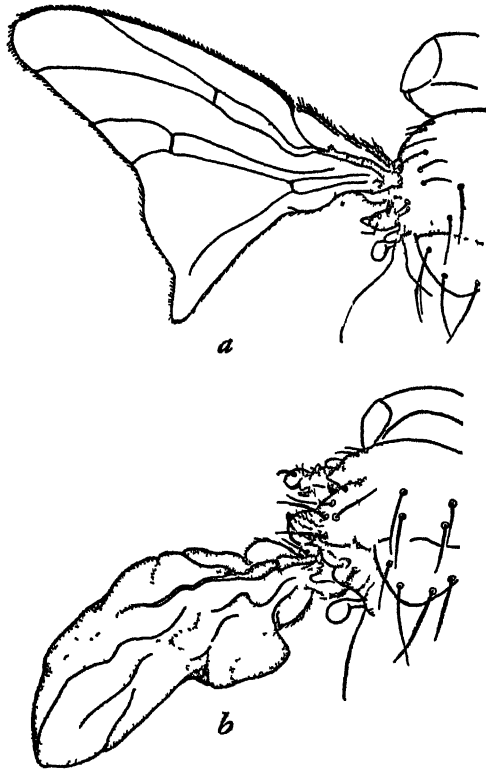


Fig. 11. *a.* Pod-II. Tetraltera-like wing. *b.* Pod-II. Mirror-image duplication of the wing blade.

The second type of reduction occurs as the result of incision along the margins of the blade (pl. 12, figs. 7, 8, 10). The veins of the wing in the region of the incision are usually broken and frequently are completely missing. In the less extreme types, the blade remains more or less normal except that varying portions of the wing blade are missing (pl. 12, fig. 10). A complete series has not been observed, because this type of wing reduction is not frequent. The more common method of reduction is that exemplified by type IV flies. In this, the earlier stages show both incision and extreme abnormality in venation (pl. 12, fig. 16). Subsequent reduction results in a vestigial-like wing (pl. 12, figs. 17-18) which has a tendency to separate into a quadripartite structure. Eight flies showed various transitional types from type I to type II*a*, four

a transition from IIa to IVa. Type IV showed transitional stages with IIIa (7 cases) and IIIb (7 cases). The final changes vary considerably: sometimes a rudimentary wing blade is left; more frequently, however, the wing is reduced to chitinized bulla-like protuberances on the thorax, or a part of the wing may be a chitinized knob, the other parts retaining wing-tissue characteristics. The flies that go through these transitional stages show individual variations.

Of the rare variants only two are pictured. The fly represented in fig. 11a has a wing which is characteristic of the tetraltera type I phenotype rather than that of podoptera. The tetraltera wing has a duplication of the posterior rather than the anterior portion of the wing. The wing of this fly has six longitudinal veins rather than five, and three cross veins. Furthermore, a portion of this wing has been reduced to a chitinized protuberance on the thorax. The second variant is a characteristic type VI as far as the anterior part of the wing is concerned, having a parapod; but the posterior part is a complete mirror image of the wing blade, including two alulae (fig. 11b).

Rapoport's "Met," which is a dominant mutant, closely resembles pod-G and pod-H in its morphology. It seems that the same types and transitions were found by him that we found in our stocks. The main difference is that our class V is found in 60 per cent of the flies of his stock. The development of the wingleg from the costa is identical. Also, the significance of the structure as a homoeotic change into a leg is realized, and some phylogenetic conclusions resembling closely those presented by Goldschmidt (1945) are drawn. The only visible difference between Met and the recessive pod stocks is a greater tendency to abnormalities of the thorax of the same type as we described in tetraltera: excrescences with strange whorl-like arrangements of the hair and, further, a higher incidence of hemithorax types. Rapoport has made a special study of the details of bristle changes. The changes observed by him seem to differ little from those encountered in our material, as our figures show. Rapoport was especially interested in analyzing his material from the point of view of determination of the metathoracic disc.¹

d. Podoptera K and podoptera M(3)124

Since the transformation of the wing into the homoeotic structure in the stocks pod-M(3)124 and pod-K closely follows that found in pod-G, the classification of podoptera phenotypes as given for this stock (p. 82) has been used here without change in classifying pod phenotypes. In table 11 the incidence of pod and the relative frequency of the types of expressivity are shown for both stocks. In pod-M(3)124 the numbers of pod females and males are equal (22.0 per cent). In both sexes the great majority of pod flies belong to class VI. The same preponderance of class VI is also found in pod-K. In this respect the two stocks differ markedly from pod-G, pod-H, and tet. The sex ratio in pod-K is females 1.3 : males 1.0. Incidence of pod is lower in the males (28.1 per cent as compared to 34.6 per cent in the females). This stock, pod-K, was originally derived from a mating with the Patterson stock ($\underline{y}$; bw; e^4 ; ro; ey). The females

¹ Addition, November, 1950: We have since found a similar dominant Pod, which is being studied.

carry attached X chromosomes and consequently contain a Y chromosome. After outcrossing with the podoptera male, it was found that the pod phenotype reappeared in F_2 and backcross males, but never in any female which did not contain a Y chromosome (for one exception see p. 244). The Y chromosome therefore appears to be essential for the manifestation of the podoptera phenotype in pod-K.

B PLEIOTROPIC ACTION

In most of the work done on the pod effect only the homocotic changes of the wing were used in recording the phenotype. In all the pod lines the genetic pod factors produce other effects of considerable variability. For some of them,

TABLE 11
INCIDENCE OF PODOPTERA IN pod-K AND pod-M(3)124

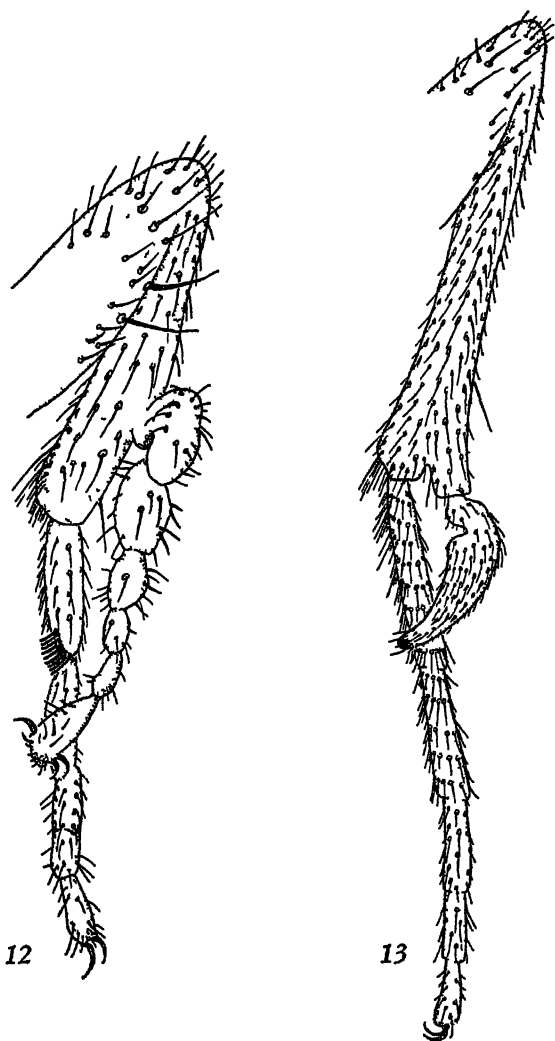
Stock	Total flies	pod							Percent-age pod
		I	II	III	IV	V	VI	Total pod	
pod-M(3)124	1,966	7	58	16	2		358	441	22 1
Females		2	9	2			206	219	22 0
Males		5	49	14	2		152	222	22 2
pod-K	2,008	36	50	10	19	1	523	639	31 7
Females	1,151	8	33	9	11		337	398	34 6
Males..	857	28	17	1	8	1	186	241	28 1

statistical data were presented in earlier morphological studies (see Vilee, 1942, pp. 149 ff.; Goldschmidt, 1945, pp. 88 ff.). All these effects turned out to be rather elusive, although sometimes a regularity was observed. The manifold effects upon form of thorax, number, position, and form of thoracic bristles, and tendency to different grades of hemithorax can hardly be called pleiotropic effects. The dorsal mesothoracic disc is not separated in early developmental stages from the wing disc. Tensions or distortions produced by abnormal development of the wing may affect the development of the thorax in a simple mechanical way, or they may upset the time or the line of separation of the two parts, or may by similar actions produce in one half of the disc direct secondary effects of the changes upon the development of the other half. The following paragraphs deal with what are considered to be real pleiotropic effects.

a. *Podoptera G* and *tetraltera*

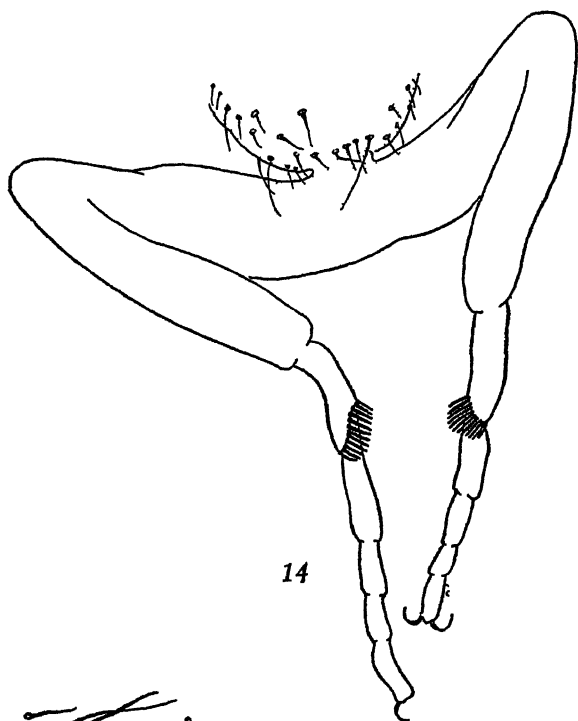
The most conspicuous pleiotropic effect found in all pod and tet lines is the effect upon the legs which was described and tabulated for pod-G in 1945. It is also found in Rapoport's Met stock. The irregularity of the effect was also described, also the correlation with the grade of wing effect. The leg effect was always sporadic in pod stocks and rather rare in the tet stocks. Occasionally an unexpected regularity appeared in tet for a generation or two: all high-grade tet flies had only the first leg crippled or absent, and this always on the

same side as the asymmetrical wing effect. But when a more exact study of this was started, it was found that the regularity had disappeared, and again the effect was completely haphazard. It seems unlikely that special modifiers

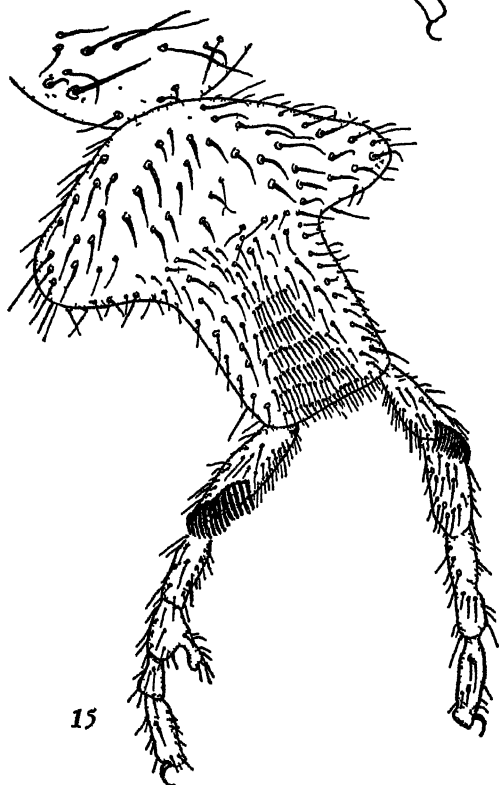


Figs. 12, 13. Two types of leg reduplications found in the special line described in the text.

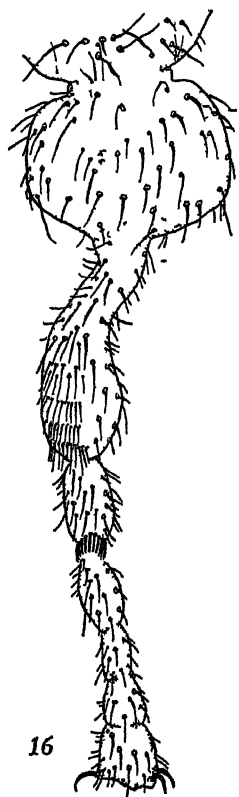
could have caused this regularity, since it again disappeared in a close-inbreeding experiment. When the influence of the Y chromosome on tet was studied, however, an indication was found that some modifiers might act specifically upon the pleiotropic leg effect without affecting any of the other manifestations of the tet effect. A tet line, to be described below, with powerful tet factors in the X chromosome was marked with first-chromosome recessives, and a crossover combination *y cv tet* was extracted. This X chromosome was



14



15



16

Figs. 14-16. Beginning of union of prothoracic legs. 15. Union of femur and tibia into broad, flat plates. 16. Complete but irregular union of the prothoracic legs.

again combined with autosomes containing tet factors, and thus a high-penetrance tet line, marked with yellow and crossveinless, was obtained. Some broods of this line showed an immensely increased leg effect. Most tet flies had abnormal legs, some of which were very extreme. Selection was ineffective. But, after outcrossing flies of this line which did not show the leg effect, and backcrossing them again, flies of one mating among about fifty showed again the extreme leg effect. In these cases very remarkable leg structures appeared

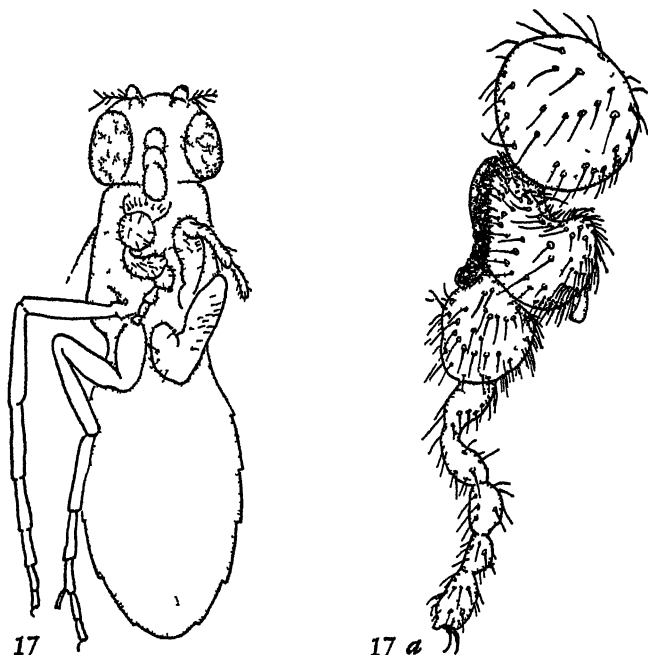
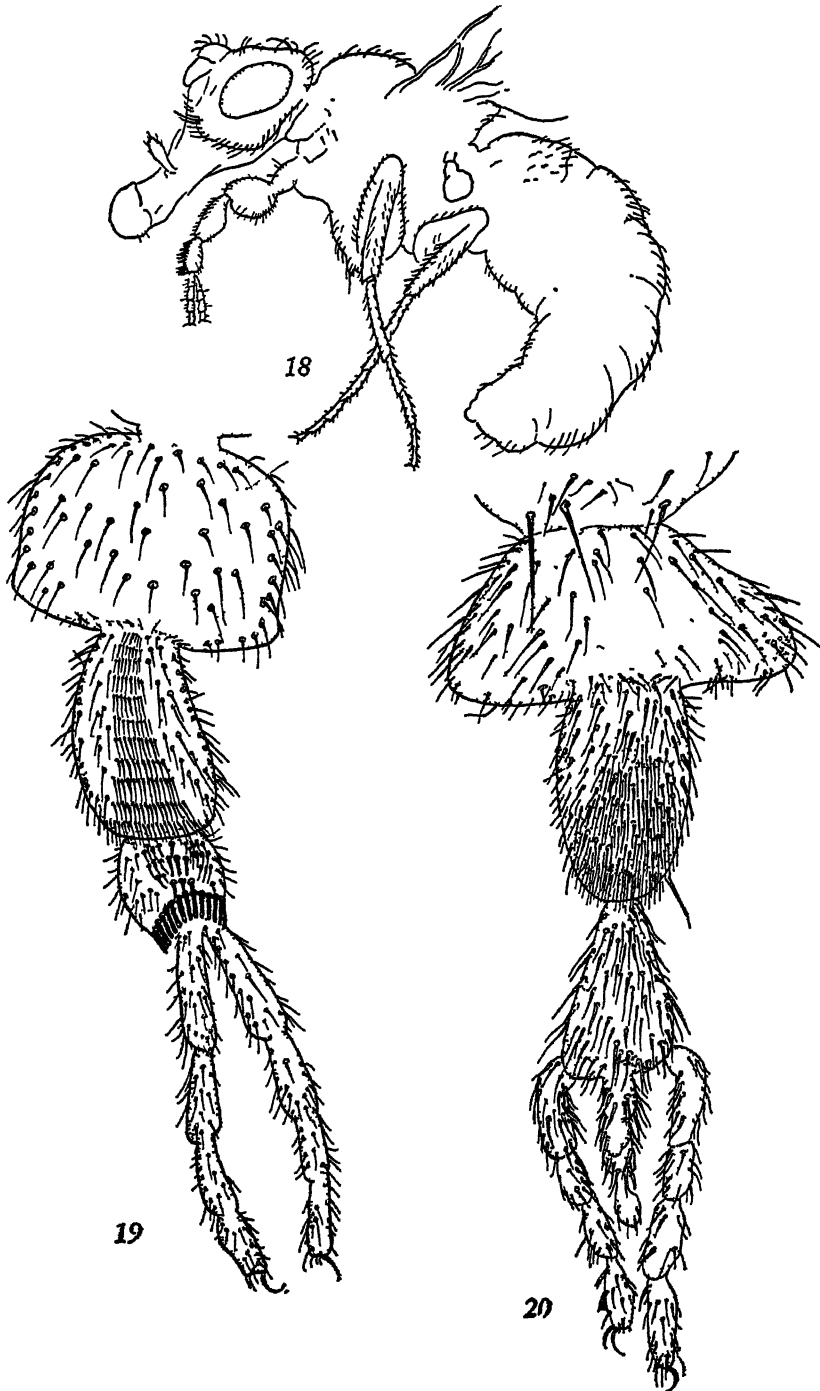


Fig 17 Similar to 16, but other legs also affected a Details of forelegs of fig. 17.

in addition to those described by Goldschmidt (1945) and below for pod-H. Legs with duplicated tarsi, or tarsi plus half of a tibia or complete tibiae and tarsi, looking like a biramous extremity, were frequent. Many times, one to four legs were short, very broad and stocky, and covered with thick spines, reminding one of some Coleopteran legs. Frequently one foreleg was missing. But the most amazing types were those in which the two forelegs were united into a single structure attached to the middle line of the thorax. In some flies the basal segments of this structure broadened into a plate, and the whole structure reminded one of the labium of an Orthopteran in many ways (see figs. 12-20, especially 20). Of course this resemblance is more or less incomplete, but one cannot help feeling that this shows how, in a single step, an organ typical for an order of insects may have originated by a single major mutation—the unpaired labium of a *Periplaneta*, for example, out of a pair of biramous appendages (see discussion in Goldschmidt, 1948a).



Figs. 18-20. Side view of fly to show position of united forelegs. 19. Details of united forelegs of fig. 18. Notice the transformed sex combs. 20. Labium-like forelegs.

The behavior of some of the other pleiotropic effects was just as irregular. The most conspicuous of these are in pod-G (not in tet):

- 1 A tendency of the normal-winged flies not to expand their wings was observed. In one generation of a continued inbreeding experiment all not-pod flies had folded wings. In the next generation a very large number showed the abnormality. In the following generations fewer and fewer flies behaved the same way, and finally the type disappeared. There was no known or observed change in the environment. This wing effect seems also rather variable. Sometimes the wings are blown up to full length but the major fold is not straightened out, so that the wing looks as if it is broken or jointed. Sometimes the wing is extended to its full length but folds up like a wet towel. The relative number of these phenotypes varied, and no selection was successful. (This occurred in lines long inbred and hence homozygous.) All this happened more than once in inbred and isogenic lines in which segregation of modifiers was not impossible but improbable. One could think of external conditions, like moisture of food, type of yeast, or crowding, as responsible factors. But the same phenomenon was never observed in heterozygotes or different stocks bred simultaneously. It may be assumed therefore that the pleiotropic effect is based upon a hypersensitivity of pod flies to unknown environmental conditions.

2. There is a tendency of the normal wings in a podoptera line to show blisters. Again, this phenotype appears sporadically, independent of the phenotype of the parents, without any rule and without apparent relation to either genetic modifiers or to environmental conditions. The following example illustrates this (table 12, p. 98).

The apparent clustering of blistered flies in the F_2 offspring of no 9635 is due to the method of tabulating, in which the many more frequent cases of normal F_1 and F_2 offspring of blistered flies have been omitted. The low frequency of the blistering effect as compared to the wingleg effect can be judged from the following count made in F_2 of the isogenic pod-G line (in the experiment recorded below in table 27): 16 bottles with 4,452 flies. Among them were 10 blistered (in 5 bottles) = 0.22 per cent. In individual bottles there were: (a) 6 in 395, (b) 1 in 40, (c) 1 in 385, (d) 1 in 306, (e) 1 in 294. In all, there were 48 pod flies = 1.1 per cent. Two of the bottles with blistered flies did not have any pod flies.

It is not worth while to add more details, as these examples already bear out the statement that the blistering effect in pod-G is an elusive pleiotropic effect of the pod genotype without any clear relation to genetic or environmental modification. It should be added that in all other lines of pod-G a similar behavior of the blistered phenotype was found. It should be very difficult to disprove that these effects might also be based upon another low-penetrance condition which by chance is present in the stock. No further analysis was undertaken.

There is reason to believe that the blistered effect of the pod factors may also be specifically affected by modifiers. A Beadex (Bx) stock had been found to produce a rather high percentage of podoptera flies. The podoptera effect

was partly due to the Bx locus. Simultaneously a large percentage of the Bx females (only a few males) had blistered wings, and this could not be separated from the Bx locus. It seems that Bx acted as a podoptera factor (in collaboration with autosomal factors) but predominantly enhanced the blistering effect.

TABLE 12

BLISTERED FLIES IN THE ISOGENIC pod-G LINE MARKED WITH $\text{In}(1)y^{px} \text{bl}, \text{In}(1)w$

(Only bottles containing blistered flies are recorded. Within the generations F_4 – F_8 there were 55 bottles, 8,981 flies without blistered flies. These comprise the normal offspring of blistered flies in F_1 and F_2 —all of which were tested—of their normal sisters and brothers and of unrelated normal broods. Thus, all F_1 and F_2 , etc., from one blistered parent, which did not again show blistered flies are recorded in the “not related” group. These negative tests for the heredity of blistering constitute the majority of matings. “Unrel.” means unrelated in the 2 preceding generations to broods with blistered flies.)

Generation	No.	Parents	Grandparents	+ flies	blist	Remarks
F_4	9370	9219 + × +	+ × +	203	1 ♀	Cousins of 9370 Unrel.
	9373	9219 + × +	+ × +	171	1 ♂	
	9487	9164 + × +	+ × +	150	1 ♂	
F_5	9520	9370 + × +	9219 + × +	136	1 ♂	Unrel.
	9635	9464 + × +	+ × +	69	2 ♂	
F_6	9675	9519 + × +	+ × +	86	1 ♀	Unrel.
F_7	10072	9868 + × +	+ × +	83	2 ♀	Unrel.
	10076	9877 + × grandfather				
		9635 bl	9635 + × bl	11	1 ♀	
	10082	9841 + × +	9635 + × +	50	2 ♂	
F_8	10091	9881 + × +	9635 + × +	150	1 ♀ 1 ♂	Unrel. Unrel., cousins of 10121
	10121	9902 + × +	+ × +	177	1 ♂	
	10122	9902 + × +	+ × +	250	1 ♂	
	10175	9876 + × +	9635 + × +	74	1 ♂	

3. Another pleiotropic effect upon the wings is the appearance of nicking and scalloping. Class I of the pod effect in tet and pod-G may show scalloping of the affected wing. This might be considered a part of the process of reduction of the wing blade in a pod wing. (The decisive criterion for calling the wing of tet or pod-G a pod wing is always the position of the wing at right angles from the body.) But the normal wing of a pod or of a + fly in a pod line also may show the nicking. Again, the frequency of the effect is rather variable within and between the lines. Many cases have been tested, but the effect has never been found to be a typical and recurrent part of the pod action. Numerically this effect is rare. For example, in one of the selected pod-G lines only 5 nicked individuals were found in 5 bottles (in 8 generations) comprising 829 flies. But there is some relation between the nicked phenotype and the pene-

trance of the pod effect—a relation which would deserve a special investigation if lines with a higher incidence of the nicking effect could be found. We found that, three times, when breeding from a nicked fly in the inbreeding experiments reported in table 1, an unusually high incidence of pod types was obtained. In F_{20} of the $T(1; 2)Bld/In(1)y^{px\ bl}, In(1)w$ inbreeding series, three unrelated broods contained nicked flies (none in F_{18}, F_{19}):

No. 11312	165+, 2 ♂ pod	1 ♀ nicked
No. 11318	221+, 8 ♀ 2 ♂ pod	1 ♀ nicked
No. 11329	205+,	3 ♀ nicked

Among these, two had a lower percentage of pod than average, namely, average 1.8 per cent, against 0.54 per cent here. (See discussion of table 1, group 4.) But no. 11318 had 4.3 per cent pod.

One nicked female of each of the 3 broods was mated to a normal brother:

		Penetrance of pod in parental brood
F_{21} No. 11558 = 11329 ²	152+, 3 ♀ 4 ♂ pod = 4.4 per cent	0.0 per cent
No. 11569 = 11312 ²	130+, 5 ♀ 3 ♂ pod = 5.8 per cent	1.2 per cent
No. 11570 = 11318 ²	93+, 5 ♀ 4 ♂ pod = 8.8 per cent	4.3 per cent

Thus, the offspring of all 3 nicked females had an unusually high penetrance of pod, although the parental broods had shown variable amounts of penetrance. There is a clear positive correlation between the parental and filial percentage penetrance.

In F_{22} of the inbreeding experiment only 6 pairs could be successfully bred (because of a heat wave). One brood was the offspring of an unrelated F_{21} (unrelated in the sense of table 12), no. 11613; one came from the high penetrance parents F_{21} no. 11558; and 4 from the highest penetrance F_{21} no. 11570. The results were:

No. 11613	unrelated	83+, 3 ♀ pod	pod penetrance 3.5 per cent
No. 11729 = 11558 ²		47+,	
No. 11740 = 11570 ²		102+, 1 ♀ pod (1 ♀ nicked)	pod penetrance 1.0 per cent
No. 11847 = 11570 ²		130+, 1 ♀ 1 ♂ pod	pod penetrance 1.5 per cent
No. 11848 = 11570 ²		134+, 1 ♀ 3 ♂ pod	pod penetrance 3.0 per cent
No. 11850 = 11570 ²		145+, 2 ♀ 1 ♂ pod	pod penetrance 2.1 per cent

Thus, the high penetrance of F_{21} was not maintained. Actually, the highest penetrance appeared in a brood not directly descended from the F_{20}, F_{21} lines.

From this unrelated line three F_{23} were bred:

No. 11759 = 11613 ²	130+, 1 ♀ pod
No. 11760 = 11613 ²	154+, 3 ♀ pod
No. 11762 = 11613 ²	150+, 2 ♀ pod

434+, 6 ♀ pod = 1.4 per cent

And again, three F_{24} were bred:

No. 11871/73 = 11760² 393+, 2 ♀ pod = 0.5 per cent

Here there was the usual irregular penetrance (see below). But the one nicked female of F_{22} bred to a brother produced:

No. 11952 11740² nicked $\times$ + 131+, 5 ♀ 2 ♂ pod = 5.1 per cent pen.

This penetrance, too, is well above that of the preceding generation; although here no high-penetrance line could be selected, every nicked female which appeared had an offspring with high penetrance of pod.

It is very difficult to explain this fact. After brother-sister inbreeding for more than 20 generations the line should be largely homozygous except for the chromosome sections involved in the Bld-translocation (always breeding $T(1; 2)Bld/In(1)y^{px\ bl}, In(1)w \times In(1)y^{px\ bl}, In(1)w$). The translocation itself cannot be responsible, for many checks showed that the pod flies were as frequently Blond as not. The most plausible explanation is therefore that a pod factor (or a higher allele of an existing one) arose by mutation, and that a pleiotropic effect of this mutant was a tendency to nicking.

A third case belonging to the same category was found when the line pod-K originated (see above: *Podoptera K*).

b. Podoptera H

A number of phenotypes were found in the pod-H stock and in inbred lines of pod-H different from those described as pleiotropic actions of pod-G. These include gynandromorphs, bristle mosaics, and rough eyes and are of special interest. (Only pod-H showed these effects, although gynandromorphs were sometimes found in pod-G.) Those which occurred in a relatively high incidence were tested for their relation to the podoptera complex, i.e., (1) pleiotropic and (or) multiple expression of the pod mutant or mutants ("multiple effects" means the visible changes that are direct or mechanical consequences of the change in the wing; "pleiotropic" effects are those upon a different region of the body), (2) a mutant separable from the pod mutants but interacting with the pod mutants to enhance or inhibit the expression of the pod phenotypes, (3) a mutant separable from the pod mutants and having no effect on podoptera action. The multiple array of phenotypes found to be a part of the podoptera complex, i.e., pleiotropic effects and multiple effects, were:

DEVELOPMENTAL ACTION	REGION AFFECTED	PHENOTYPIC EXPRESSION
Primary effect		podoptera tetraltera-like
Multiple effects	dorsal mesothorax	hemithorax; abnormal bristles on thorax, short wing, blistered wing, plexus-like wing
Pleiotropic effects	dorsal metathorax	tetraltera-like
	ventral thorax	deformed legs
	head	rough eyes
	abdomen	etched abdomen
Chromosome elimination	all regions	gynandromorphs, haplo-IV mosaics

The multiple effects affecting the thorax of the pod fly were not so common in pod-H as in pod-G and tet. The bristle pattern was usually quite normal, or if it did show abnormalities, the usual effect was a Minute-like reduction of all the thoracic bristles on the podoptera side, rather than a duplication of certain of the macrochaetae. The incidence of hemithorax flies was likewise not as great in pod-H as in pod-G. The pleiotropic phenotypes, on the other hand, were very diverse, affecting every portion of the body with the possible

exception of the prothorax, and including some types not found in the other podoptera lines. Table 13 gives the incidence of the various types as found in various sublines of pod-H.

1. *Leg modifications*.—Among the effects that are strictly developmental, i.e., leg, wing, and eye abnormalities, the leg modifications were studied in detail. Text fig. 21 shows some of the modifications. The leg pictured in fig. 21b



Fig. 21. Leg abnormalities in pod-H. Explanation in text.

is the characteristic type of abnormality, although the partially duplicated leg was not infrequent (fig. 21a, c). Complete duplication giving 7 (fig. 21d) or even 8 legs was very rare. Of the 490 flies analyzed for podoptera (see section on Morphology and Variability), 123 also had leg abnormalities (table 14). Compared to the expression of this effect in pod-G, pod-H has a lower incidence of leg effects. This decreased incidence is expressed in every class as well as in the total numbers.

Among 105 flies with both podoptera and leg effects, 75 flies with only one wing affected had abnormal legs on the same side, and 30 showed wing effects on one side and leg effects on the other. With a 2×2 contingency test, $\chi^2 = 17.44$ per cent; therefore, it may be concluded that the leg effect is not independent of the podoptera effect.

TABLE 13

INCIDENCE OF PODOPTERA AND FLIES WITH OTHER PLEIOTROPIC EFFECTS IN pod-H LINES

Line	Total flies	podoptera			gynan- dro- morphs	Wing										Haltere		Thorax		Leg		Eye		Abdomen				
		Total	Per- centage	Total		Total		Small		Plexus-like		Blistered		Cut		♀	♂	♀	♂	♀	♂	♀	♂					
						♀	♂	♀	♂	♀	♂	♀	♂	♀	♂													
Inbred Fla. Iso. '89..	2,875	42	1.46	..	1*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	♂	♀	♂	♀	65	16	
Inbred pod-H (high pod).....	17,249	599	3.47	72	24*	6*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Inbred pod-H (high gyn).....	9,776	255	2.61	232	23	8	19	4	2	1	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Replacement XpYp2p3p.....	5,565	135	2.43	103	6	..	2	..	1	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Isogenic (1st 7 gen.)	4,333	115	2.65	16	20*	16*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Isogenic (Inbred)...	1,323	62	4.69	1	17+	15*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Z.....	41,121	1,208	2.91	424	91*	45*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

* Not recorded in separate classes.

TABLE 14

COMPARISON OF INCIDENCE OF pod FLIES WITH ABNORMAL LEGS IN pod-G (GOLDSCHMIDT, 1945) AND pod-H
(Numbers in parentheses indicate the number of flies with abnormal legs.)

	Class I	Class II	Class III	Class IV	Class V	Class VI	Bilateral
pod-G 379 (132).....	63 (4)	78 (50)	71 (30)	30 (14)	28 (15)	78 (30)	31 (12)
pod-H 490 (123).....	39 (3)	167 (54)	149 (35)	32 (9)	9 (1)	53 (8)	61 (13)

The third leg is affected more frequently than the second or the first (table 15). These data would suggest that the critical period of the podoptera effect would be between the time of differentiation of the second and third legs, for in normal development, differentiation occurs earlier and is completed sooner in the first leg discs than in the second, and earlier in the second than in the third (Auerbach, 1936).

TABLE 15
PODOPTERA H WITH ABNORMAL LEGS

Leg affected	Total number of pod	One side pod	Bilaterals
One first leg	7	7	..
One second leg	18	18	..
One third leg	70	60	10
First and second on one side	..	..	..
First and third on one side	8	7	1
Second and third on one side	12	12	..
First, second, and third on one side	1	1	..
Second on both sides	3	2	1
Second left; third right	3	3	..
Third left; first, second, third right	1	..	1

TABLE 16
INCIDENCE OF PODOPTERA AND GYNANDROMORPHS IN FIVE INBRED LINES OF pod-H

Line	Total flies	Podoptera		Gynandromorphs		Fertile gynandromorph crosses
		Total	Percentage	Total	Percentage	
1st 4 generations ..	1,732	75	4.33	4	0.23	1
1073	2,275	114	5.01	4	0.18	2
1080	2,381	65	2.73	29	1.22	10
1081	1,878	85	4.53	5	0.27	2
1083	2,174	24	1.10	7	0.32	2
1084	6,809	236	3.46	23	0.34	9
Σ	17,249	599	3.47	72	0.42	26

2. *Chromosome elimination*.— a) Elimination of an autosome: Flies with Minute-like bristles on one half of the thorax occurred rarely (0.10 per cent) but consistently throughout the experiment. Most frequently this was a part of the multiple expression of the podoptera effect, for the flies were podoptera for the same side. One fly (heterozygous for the fourth-chromosome mutant shaven) however, was shaven on one side of the body and had normal bristles on the other side. As mosaicism of this sort is a characteristic expression of the haplo-IV condition (Sturtevant, 1929) it may be postulated that the Minute-mosaic effect sometimes is the result of elimination of the fourth chromosome.

b) Gynandromorphs: Chromosome elimination was more easily recognizable, and therefore more striking, in the X chromosome, for here gynandromorphs

were produced. Even in the preliminary experiments a number of gynandromorphs were observed. In the first inbreeding experiment (table 16) the incidence was 1.22 per cent in line 1080, although the average incidence was only 0.42 per cent. After inbreeding gynandromorph "females" to podoptera males by mass matings for a number of generations, the incidence of gynandromorphs was increased to more than 2 per cent. Five generations of inbreeding by single-pair matings of gynandromorph "females" by normal males from this stock gave an average incidence of 2.52 per cent gynandromorphs (table 17).

All the gynandromorphs were mated every time, but as would be expected, there was a high degree of sterility (table 16, cols. 5, 7). Whenever one was

TABLE 17
INCIDENCE OF PODOPTERA H AND GYNANDROMORPHS IN A HIGH GYNANDROMORPH LINE

Generation	No. of flies	Podoptera		Gynandromorphs	
		Total	Percentage	Total	Percentage
F ₁	389	9	2.32	7	1.79
F ₂	293	12	4.06	10	3.14
F ₃	408	12	2.94	16	3.92
F ₄	910	20	2.19	19	2.08
F ₅	619	14	2.26	15	2.42
Σ.....	2,619	67	2.52	67	2.52

bred, matings of phenotypically normal brothers and sisters were made at the same time. Of nine pairs of such matings the gynandromorph × phenotypically normal gave 3.50 per cent pod among 417 flies, while the normal × normal gave 2.64 per cent among 962 flies.

Because of the low incidence of both podoptera and gynandromorphs, a similar comparison could not be made for podoptera vs. gynandromorph crosses. However, in a later experiment, after a line had been selected for podoptera and gynandromorphs for 26 generations, sibling matings gave:

- (1) gynandromorph × normal, 2.20 per cent podoptera among 998 flies
normal × normal, 1.98 per cent podoptera among 1,171 flies
- (2) gynandromorph × normal, 3.00 per cent podoptera among 1,031 flies
podoptera × normal, 3.43 per cent podoptera among 729 flies

The incidence of gynandromorphs in these crosses was:

- (1) gynandromorph × normal 1.91 per cent
normal × normal 1.57 per cent
- (2) gynandromorph × normal 3.59 per cent
podoptera × normal 1.51 per cent

Although the differences in incidence of podoptera (and gynandromorphs) among the offspring of phenotypically different parents, though brothers and sisters, is not statistically significant, these data suggest the probability that the expression of the podoptera and gynandromorph phenotypes is related in some complex manner.

The inbred lines (table 16) varied considerably in the incidence of gynandromorphs. Likewise, a low incidence of gynandromorphs was exhibited by the lines with the highest incidence of podoptera, except line 1083, in which other factors were acting to inhibit the expression of pod (see below). Therefore it appeared as if there was a negative relationship between the incidence of podoptera and gynandromorphs. This was tested by a correlation coefficient test. Because of the low and variable incidence of podoptera and gynandromorphs in a single mating, all the progeny of a single generation were combined (table 30 under heading, "pod $\times$ pod, strict selection"). The correlation coefficient for all of the lines combined is $r = -0.48$ (16df), which is significant. In line 1080, which had the highest incidence of gynandromorphs, $r = -0.44$ (11df), which is probably biologically significant. This hypothesis was further tested with data, from an inbreeding experiment, in which the incidence of gynandromorphs and podoptera was about the same, i.e., 2.5 per cent. In this case, $r = -0.41$ (25df), which is statistically significant. Therefore it may be concluded that there is a negative correlation in the expression of podoptera and gynandromorphs.

Although a correlation test gives no indication of the processes involved in the expression of the two phenotypes, consideration of the experimental data suggests that one or more of the following explanations may be correct:

(1) The podoptera and gynandromorph enhancers are separate and segregating, so that selection for podoptera fixes podoptera enhancers, and vice versa.

(2) Some of the modifiers may be inhibiting for one phenotype and enhancing for the other.

(3) The genotypes of both are the same, but embryological processes are such that development is either toward podoptera or toward gynandromorph expression. This explanation seems to fit the facts best and to agree with other facts to be presented later.

c. Podoptera K

In addition to the types rough eye (ro-1) and notched wing (no-1) already mentioned, several others were found in the stock pod-K. They were: small or kidney-shaped eyes (not allelic to eyeless), spread wings, deformed legs, taxi-like, curved, or blistered wings, and absence of posterior vertical bristles (pvm). Only the pvm phenotype occurred frequently and regularly enough to be included with ro-1 and no-1 in the morphological study of the stock.

A tabulation of the relative frequencies of the types is presented in table 18. Rough eye appears in 100 per cent of the flies; the other characters occur with variable frequency. No effort was made to select lines with complete penetrance for any of the pleiotropic effects.

Because of the difficulties encountered in the localization of the pod factors in this stock (p. 188), it cannot be decided with certainty whether these effects are actually due to pleiotropic action of one locus or to a number of separate genetic factors in the second chromosome. The following facts, however, strongly suggest pleiotropic action of a single locus: (1) After outcrossing, pod flies never appear without rough eye. (2) Notched wing and pvm always segre-

gate together with rough eye after outcrossing. Without very accurate localization of all of the supposedly pleiotropic effects of the pod factor, however, the presence of a group of closely linked genetic factors rather than that of a single locus cannot be excluded on the basis of the data presented.

The Y chromosome again appears to be essential for the manifestation of the pleiotropic effects of the pod factors in the females. Although the different

TABLE 18

INCIDENCE OF ROUGH-LIKE (ro-l), NOTCHED-LIKE (no-l), AND ABSENCE OF POSTERIOR VERTICAL MACROCHAETAE (pvm) WITH AND WITHOUT PODOPTERA IN pod-K

Phenotypes mated	ro-l		ro-l no-l		ro-l no-l pvm		ro-l pvm	
	with pod	with-out	with pod	with-out	with pod	with-out	with pod	with-out
pod × pod.....	1	37	1	97	8	119	6	25
ro-l no-l × ro-l no-l.....	2	3	1	9	9	90	2	37
Σ.....	3	40	2	106	17	209	8	62

types occurred in both the males and females ($\underline{XY}$) of the pod-K stock, they were never observed in females after outcrosses which resulted in females with two X chromosomes and without the Y.

d. Podoptera M(3)124

In the stock pod-M(3)124, deformed legs, blistered wings, and irregularities in wing venation were found in many of the broods. These abnormalities have been reported as being characteristic of Minute stocks (Brehme, 1941). They are, therefore, pleiotropic effects of the M(3)124 mutant, which is simultaneously an important podoptera factor. Another phenotypic variant frequently encountered in pod-M(3)124 flies is a wing with irregular venation of about one-half to two-thirds normal length, but in the normal position. Since this type of wing also occurs in the stocks pod-G and pod-II, it might be considered to be an effect of pod factors other than the Minute rather than a result of specific action of the Minute.

C. SPECIFIC MODIFICATION OF PLEIOTROPIC ACTION

a. The action of Dichaete

In this section a group of facts will be presented showing that in the presence of certain dominant markers one or the other of the pleiotropic effects of the pod factors is specifically enhanced, e.g., although the penetrance of the pod character is not increased, the leg effect is greatly increased.

The first of these to be mentioned is the action of Dichaete (D) upon blistering. Dichaete was not used extensively in our experiments, because the spread wings of Dichaete cannot be properly distinguished from a symmetrical, class I pod effect. But it was observed that when Dichaete was crossed to a

pod-G line with low penetrance of blisters, F₁ flies heterozygous for D and pod had a large percentage of blistered flies, and flies containing the other balancer chromosome were always normal. For example:

pod × D/GI	195 not blist (incl. 80 D)	31 ♀ ♂ D blist
pod × D Sb/In(3)R Payne	112 not D not blist	100 ♀ ♂ D Sb blist

Actually, the Dichaete stocks have a very slight tendency to develop blisters, which may be due to the Dichaete chromosome. This tendency acts as if it were allelic to pod, as far as the blistering condition of the pod factors is concerned. But, as no homozygous blistered strain could be extracted from pod, it is more probable that the pleiotropic blistering effect of pod enhances a similar action of the Dichaete chromosome (or vice versa) to a high-grade penetrance.

b. The action of Hairless

Hairless (H) was combined with podoptera and tetraltera in such a way that at least the second-chromosome pod or tet factors were homozygous (details below). In these combinations, both with pod-G and with tetraltera, a remarkable effect was found. In tetraltera, a considerable number of the flies containing H showed abnormalities of the wings which looked exactly like the tet classes II-III but with the wings in normal position. In addition, the legs were strongly affected, much more so than ordinarily. The effect resembled different grades of the mutant dachs and was sometimes so extreme that only leg stumps were left. Podoptera flies combined with Hairless showed also more frequent leg effects and more extreme ones than usual. To give only one example: in one generation of this combination, 48 among 380 flies showed all these features = 13 per cent, i.e., many times the penetrance of the wing effect in pod. Hairless thus acts upon the expression of podoptera as a modifier. But the interesting point is that the different pleiotropic effects of pod (and tet) are selectively influenced by the presence of H. The most important effect of pod, the wing transformation, always begins with the fixation of the wing at right angles. H does not affect this at all, and as this character was always used to differentiate a pod wing from a nonpod wing, the penetrance of pod as per definitionem is not affected. But the wing in normal position and therefore not recorded as pod can show some of the other changes of the type of the first three pod classes. Another pleiotropic pod effect is blistering found with lower penetrance than the typical wing effect of pod, and this is enhanced by Hairless. In one pod line with H which was bred for a long time blistering had reached a high penetrance.

This "fractionated" modifying effect may be one of the many specific features of the pod case. The possibility cannot be excluded that H is an allele of one of the pod factors which collaborates to produce the pod effect. "Allele" might have a special meaning in this case. Actually, a considerable group of comparable facts was found. They will be presented in the section on genetics.

3. SUMMARY OF PART I

1. The podoptera group of "mutants" (abbr.: pod) of *Drosophila melanogaster* is so widely spread among *Drosophila* stocks that one might say that it is ubiquitous. The usually very low penetrance, even in homozygous condition, is responsible for the little attention paid to it.

2. Podoptera is a collective name for a group of different hereditary strains in which the wings are transformed into leglike, three-jointed appendages of great variability in detail, including transitions to almost normal wings.

3. Pod lines breeding true to a definite percentage of pod flies, characteristic for the different lines, may be extracted from different wild-type as well as laboratory stocks. Most stocks which were studied by pair inbreeding contained pod flies as a hereditary condition. We may consider this effect to be practically ubiquitous; it may actually be the most frequent aberrant type in *Drosophila*.

4. Five podoptera lines were isolated for detailed analysis, each one characterized by special genetical and phenotypical features:

a) tetraltera. This name was retained, as the type had earlier been described by the senior author, erroneously, as a simple mutant. It is morphologically different from the other pod types so far as the entire wing is transformed into a haltere-like structure (with all transitional stages). But the low grades of tet greatly resemble the pod types, and the highest grade is a wingleg as in pod, although developed from the entire wing disc. Tet exists in lines of different penetrance, from a fraction of 1 per cent to 100 per cent.

b) podoptera G. In all podoptera types only the costal part of the wing is transformed into a wingleg (with all intermediate stages); the rest of the wing shows typical transformations. Pod-G was isolated from a mutant line used formerly for experiments on spontaneous mutation. It has less than 1 per cent penetrance in ordinary lines, 2-4 per cent in isogenic lines.

c) podoptera H. This was isolated from a Florida stock. It is morphologically similar to pod-G, but it has an average penetrance of 2.5 per cent. Pleiotropic effects which are specific for pod-H are the tendency to produce mosaics and gynandromorphs (2.5 per cent).

d) podoptera K. This originated in a mutant stock (dominant svr^{Poi}) and has the following distinguishing features: (1) It is always associated with rough eyes and scalloped wings, and the females show the pod character only in the presence of an extra Y chromosome. (2) It is further characterized by the preponderance of the highest classes of expression. Penetrance of pod in this stock fluctuated around 30 per cent.

e) podoptera M(3)124. This was isolated from the Minute 124 stock. It has an average penetrance of about 15 per cent. It resembles pod-G, except for special features due to the presence of the Minute mutant.

5. Additions are made to the previously published morphological analysis, emphasizing especially variants and the tendency to duplications in the costal regions of pod-G and pod-H, and in the posterior wing cell of tet. The classifi-

cation of morphological types used in the present studies is presented. Some very remarkable leg abnormalities of evolutionary significance are discussed.

6. All pod lines show pleiotropic actions in a part of the individuals, namely, blistering, scalloped and unexpanded wings, and also different anomalies of the legs. The specific pleiotropic effects in pod-H and pod-K were mentioned in point 4. A special study is presented for blistering, scalloping and the specific features of pod-H, with emphasis upon the production of gynandromorphs. It is noted that some of the pleiotropic effects are also characteristic of many Minutes.

7. Some dominants affect these pleiotropic actions specifically and individually as described for the action of Dichaete and Hairless.

PART II. PENETRANCE AND EXPRESSIVITY

1. PENETRANCE AND SELECTION FOR PENETRANCE

Pod and tet lines were isolated showing a definite but variable penetrance, which was analyzed in different ways, of course under identical conditions.

The records on penetrance must be regarded as minimum numbers. Flies with abnormal wings have a tendency to stick to walls or to food, or to fail to emerge completely from the pupal case. Thus, the numbers of the higher classes especially may be too low, but as all work was done under constant conditions (25° C. and standardized food, etc.), the error will be of the same magnitude everywhere.

A. TETRALTERA

The lowest penetrance line of tet is not suitable for a study of penetrance, since, under standard conditions, this is almost nil. Another line, with relatively high penetrance, was kept for years by mass selection of phenotypically tet flies. At the outset of the experiment the penetrance of tet in this line was tested by pair breeding of tet flies. Under standard conditions the following penetrance values were found in 9 matings: 46.8, 51.2, 53.3, 71.1, 71.4, 73.8, 80.0, 88.0, 88.0 per cent; i.e., all very high values. Simultaneously, a mass culture produced by mating a number of class V tet flies gave a 50 per cent penetrance. We mentioned that the first line to be isolated in 1934 had only about 5 per cent penetrance, even with selection of tet phenotypes. In the course of the years, up to later mass selection, tet factors must have originated by mutation and increased the penetrance by new multiple-factor combinations to the level found when the following experiment began.

From the above series of broods, lines were started beginning with different parents, i.e., $+\times +$, $\text{tet} \times \text{tet}$, $\text{tet} \times +$, and were inbred in different ways for 5 generations. This is the set of broods part of which was used for the work already reported; it now will be studied for penetrance.

The curve in fig. 22 shows the penetrance in 195 broods having 17,656 flies, i.e., 8,053 normals and 9,603 tet phenotypes, M penetrance = 56.7 per cent. The average brood size was 90, showing that the viability is not very high in the selected line. The individual penetrance values have a tendency to lie near some simple ratios. The curve substantiates this impression. It is clearly not a simple curve of variation as would be expected if it were due to nonhereditary modification. The curve shows the results of studying a mixture of genetically controlled penetrance values with peaks for the ratio of normal to tet phenotypes (= penetrance) near 3 : 1 (25 per cent), 2 : 1 (33 per cent), 1 : 1 (50 per cent), 1 : 2 (66 per cent), 1 : 3 (75 per cent), possibly 7 : 1 (12 per cent) and 1 : 7 (88 per cent). If these values are significant, penetrance in this line is based upon the interaction of a small number of multiple factors, with further modifiers to account for the ratios. We shall see that the percentages are the result of factorial combinations, but that the ratios have only a chance resemblance to Mendelian ratios.

Under the assumption we should expect: (1) The offspring of tet phenotypes should have a higher penetrance than that of normal phenotypes. (2) Con-

tinued selection of normals should quickly decrease the value for penetrance. (3) Continued selection of tet phenotypes should quickly increase penetrance. (4) If no linkage is involved, very high penetrance lines as well as normal lines should be extracted quickly. With linkage of several factors and crossing over, still other homozygotes should be isolated by selection. (5) Selection from high-penetrance broods should keep up the degree of penetrance, even increase it. The tests were made in 4 separate lines of the origin reported above. Line A started with a pair of normal phenotypes ($= +$); Line B with a pair of normal $\times$ tet V; Line C with a pair tet V; and Line D with a pair tet bilateral.

Table 19 shows the penetrance of matings $+ \times +$, $+ \times$ tet and tet $\times$ tet in these lines.

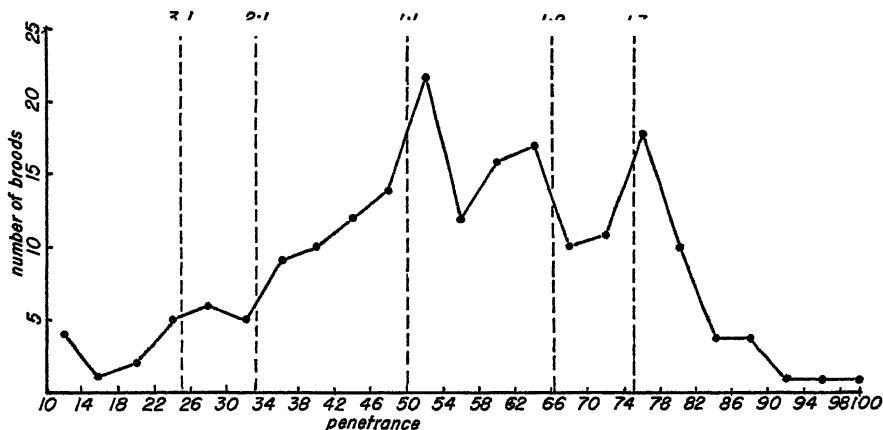


Fig. 22. Penetrance of tetralters.

The data indicate that: (1) The offspring of tet parents have a higher penetrance than that of their $+$ brothers and sisters. This is clearly visible for the total as well as for the individual lines B, C, D. (2) The penetrance of tet in offspring of parents $+ \times$ tet in all lines is rather irregular; the values are comparable to those of the offspring from tet $\times$ tet, if the one unusual brood (only one in this test, namely, third horizontal row, center) is disregarded. (3) Line A, derived from a normal pair, has relatively low penetrance whether bred from $+ \times +$ or tet $\times$ tet. (4) There is a clear influence upon the results of the type of the pair selected as original parents. In all three vertical columns for penetrance the lowest penetrance is found in the line started with a normal pair, the highest in that started with a tet pair, and intermediate in the offspring of an original pair $+ \times$ tet. Among the offspring from originally tet $\times$ tet the bilateral pair is again superior to the asymmetrical one. These results require that, on the average, tet phenotypes contain more factors or more potent ones for production of tet; further, that the greatest number of factors or more potent factors are present in bilaterals; further, that normal individuals also contain tet factors and are not segregants for normal alleles but segregants for fewer, or less potent, tet factors. This again means that there are tet factors which in homozygous condition produce only a small percentage of penetrance.

(There is never a completely normal brood in this line; the lowest individual penetrance was 11 per cent.) Homozygous combinations of more or more-potent tet factors also have incomplete penetrance, increasing with the number or the potency of the tet factors. The results from the $+$ $\times$ tet crosses might imply a dominance of the tet factors. But it is more probable that the high penetrance in these crosses is based upon the great probability that normal flies in these selected lines are minus individuals of high-penetrance genotype. The parallel trend, in the vertical column, for these crosses with that of the two others agrees with that interpretation.

TABLE 19
PENETRANCE PARENT—OFFSPRING IN SELECTED tet LINES

Line	Parents $+$ $\times$ $+$			Parents $+$ $\times$ tet			Parents tet $\times$ tet		
	Number		Per-centage pene-trance	Number		Per-centage pene-trance	Number		Per-centage pene-trance
	$+$	tet		$+$	tet		$+$	tet	
A $+$ $\times$ $+$	652	413	39	176	203	54	396	147	27
B $+$ $\times$ tet.....	2,375	2,123	47	446	655	60	1,667	2,267	57
C tet $\times$ tet.....	594	718	55	23	100	81	655	1,100	63
D tet bilat.....	312	415	57	...	...	..	373	993	73
Total.....	3,933	3,369	46	645	958	60	3,291	4,507	58

The second and third problem of this test was continued selection for normal and tet phenotypes. Table 20 contains the data.

As we were dealing with a long-inbred and selected line, and as all other facts indicated that not very many factors could be involved, selection was tried for only a few generations. The table shows very irregular penetrance for selection of normals starting with normals or $+$ $\times$ tet (lines A, B). This irregularity is still more conspicuous if we look at individual broods. Thus, in line B the individual penetrance values are: Of F_2 , 24, 32, 44, 45; of F_3 , 39, 68, 68; of F_4 , 36, 54, 72, 78; of F_5 , 19, 30, 41, 62, 70. This shows a majority of medium and lower values but includes some high ones also. The data indicate that the phenotypically normal individuals bred in selection experiments were a mixture of individuals with genetic factors for low penetrance and minus individuals of high penetrance constitution (neglecting whatever additional environmental variation there is).

In the lines C and D, in which the original parents were tet, asymmetrical (viz., bilateral), selection for normal phenotypes is clearly effective, indicating

that the tet parents had either been heterozygous for some tet factors or had been members of different genetic penetrance groups, e.g., one parent genetically low, the other high.

Although selection of normals has a great probability of acting upon different genetic situations—e.g., genetic low penetrance by absence of the high

TABLE 20
CONTINUED SELECTION OF NORMAL AND tet PHENOTYPES IN DIFFERENT LINES
(LINES A-D DESCRIBED IN TABLE 19)
(n = number, p = penetrance)

Line	F ₁		F ₂		F ₃		F ₄		F ₅	
	Number	Percentage penetrance	n	p	n	p	n	p	n	p
a. Selection for normal										
A										
+ × +.....	233	57	...	..*	147	13	320	47	365	13
B										
+ × tet.....	333	63	324	40	341	54	378	53	345	40
C										
tet × tet.....	...	..	...	..	256	70	171	61	314	41
D										
tet bilat.....	...	..	103	60	217	53	174	50	70	44
b. Selection for tet										
B.....	...	..	278	47	358	67	69	83	86	66
C.....	157	76	407	43	465	68	35	70	...	..
D.....	163	63	238	64	434	69	300	84	452	66

* Not recorded.

factors, or low penetrance caused by heterozygosity or by selecting minus individuals of any genetic constitution—selection for tet types will more easily select genetic factors for penetrance. This positive result should increase in the order of the original parents + × tet → tet × tet → tet bilat. But only the last group agrees with this expectation. The other two are rather irregular, although a high average penetrance is maintained. This could be interpreted to mean that only a few tet factors are involved.

The expectation that most normal and tet individuals might be genetically of high or low penetrance may be tested by comparing cousin broods, i.e., different brother-sister matings from one pair of parents. If the supposition

is correct, there should be found sometimes identical and sometimes different penetrance among a set of cousin broods, whether they are bred from normals or from tet, and also high or low penetrance with either parents, or an irregular mixture. Therefore, all such groups from the largest line B were assembled. Table 21 shows the results.

Within the limits of the experiment the results confirm the expectation.

TABLE 21
PENETRANCE IN COPARENTAL SIBS OF tet

Group	Sib no.	Brother-sister matings: percentage of penetrance		
		+ × +	+ × tet	tet × tet
1 All high penetrance	1	68	..	61
	2	70	..	70
	3	72, 79	..	63, 80, 70
	4	65	..	82
2 All more or less low	1	39	..	56, 38
	2	39	52	35
	3	30	..	29
	4	14	..	19
	5	40	..	41
	6	..	..	25, 22, 31, 24
3 More tet than normals	1	56	..	52, 73, 87
	2	52, 67	..	80
	3	52	..	72, 78
	4	35	..	61
4 More normals than tet	1	68	..	61
5 Irregular	1	61, 32, 24	52, 48	47, 49
	2	44, 54	28, 63, 74, 77	65

The fourth test mentioned above (p. 110) was to establish lines of different penetrance by quick selection. This was successful only in one case, tabulated below (table 23) for bilaterals. But while the experiment was in progress a 100 per cent penetrance line was obtained after crossing. This made possible a more direct genetic attack upon the problem, and therefore the selection experiments were abandoned.

The fifth postulate was that selection from high-penetrance broods should be successful. For one of the lines the data are assembled in table 22.

We consider as high a penetrance of more than 66.7 per cent (i.e., 2 tet: 1 normal) on the basis of the penetrance curve in figure 13. If we are dealing with relatively few additive tet factors, a positive correlation of paternal and filial penetrance should be most frequently found at the extreme ends of the series, i.e., very high and very low penetrance. But, since obviously much overlapping

between the genetic effects occurs, high-penetrance broods may sometimes be plus variants of a lower mean penetrance. The table shows, in a large number of cases, the positive correlation (11). There are a few (5) borderline cases (recorded as negative) with values between 50 per cent and 66 per cent which might be considered minus variations of the same type. In 6 cases the correlation is completely negative, with filial penetrance as low as 23 per cent. Two of these cases came from the same grandparental pair of low penetrance, which shows that the parents were high variants of a lower penetrance genotype.

TABLE 22
CORRELATION BETWEEN PARENTS FROM HIGH-PENETRANCE BROODS AND OFFSPRING;
MATINGS ALWAYS tet $\times$ tet

No.	Parental		Filial broods		Remarks
	Individuals	Percentage of penetrance	Individuals	Percentage of penetrance	
1.....	90	73	58	80	
2.....	83	72	69	84	F ₃ 66
3.....	139	73	103, 110	72, 78	F ₃ 25, 23
4.....	68	75	135, 33	80, 64	F ₃ 70
5.....	88	68	21	75	
6.....	117	70	49	29	
7.....	95	71	75	61	
8.....	49	71	92	51	
9.....	74	83	97	70	
10.....	81	84	86	67	
11.....	107	72	60	33	} From same grandparents
12.....	110	78	72	23	
13.....	135	80	53	70	
M.....	...	74	..	66	
M pos. corr.....	...	76	..	76	
M neg. corr.....	...	73	..	46	

The two F₃ recorded for no. 3 are difficult to understand. Since no further analysis was made, judgment must be suspended.

The five generations of selection for bilaterals (mentioned above) were not included in this table. However, the bilateral effect gives a clear indication that genetic high penetrance has been selected, and the data bear this out, as table 23 shows. This is clearly a case of successful extraction of a high-penetrance line with only few minus broods.

If we now return to the curve of penetrance of the selected line (fig. 22) we come to the following interpretation: Penetrance is determined both by genetic composition and processes of embryological determination. The latter means that the genetic factors for the tet effect contained in this line, whether they are alone or in the highest homozygous combination which could be selected, do not produce a 100 per cent tet effect. The variation in some embryonic process in relation to a threshold condition produces a certain percentage of normal flies. The genetic situation is clearly the presence of a

number of multiple factors with additive (or multiplicative) action, which cannot be very large for each one. The favored ratios (peaks of the curves) resulting from both actions look like simple Mendelian ratios 1 : 1, 2 : 1, etc., only by chance, because the added effects of the different possible genetic combinations upon penetrance happen to vary around 33, 50, 66, 75, and 80 per cent. More than one factor may be present in individual chromosomes, thus, further values may be produced by crossing over. I suspect that the lowest values which could not be selected for in this line are the result of crossing over (of course combined with phenotypic variation).

TABLE 23
CORRELATION BETWEEN PARENTS FROM HIGH-PENETRANCE
BROODS AND OFFSPRING IN FIVE GENERATIONS
MATING BILATERALS OF tet

Generation	Individuals	% penetrance
F ₁	102	89
F ₂	163	63
F ₃	23	87
F ₄ a.....	81	80
b.....	82	79
c.....	121	95
d.....	16	100
F ₅ a.....	144	49
b.....	44	75
c.....	79	89
d.....	46	76

The correctness of this interpretation is proved by the existence of very low penetrance lines and 100 per cent penetrance lines. The former were not used much for selection work, because this had already been done for low-penetrance pod lines (see below). Their origin is the same as that of the line under discussion, all lines being derived from a single individual found by the senior author in 1934. The low line was one of the stocks which had not been selected for years. Since tet flies are less viable and fertile than their normal sibs, in the absence of selection for tet, a constant selection both for minus modifiers and for heterozygosity of strong tet factors takes place, and finally the lowest penetrance line which has no more selective disadvantage is isolated. Only two small experiments were carried out with two lines which showed hardly any tet in the stock bottles. The first showed clearly that this stock was still heterozygous for factors producing only a low effect, as table 24 shows. A low and a very low line were separated before the experiment came to an end because all matings tet × tet were sterile.

The result of the second experiment is shown in table 25. This line bred true for a very low penetrance. Only one (F₁) brood from a brood with 1.3 per cent penetrance showed a significantly higher penetrance. This probably means that chance selection of modifiers, or possibly mutation, had occurred.

The other fact in agreement with the general interpretation was the establishment of a 100 per cent tet line (penetrance = 100) by introducing a new tet factor into the high-tet line by crossing with the third-chromosome Minute M(3)124. In the sixth generation the 100 per cent line was isolated in which the X chromosome carried the new tet factor (not an enhancer). Details are given below, in the section on tet-100.

TABLE 24
SELECTION IN LOW-PENETRANCE tet LINE

Generation	Matings + × +			Matings tet × tet		
	Normals	tet	Percentage of penetrance	Normals	tet	Percentage of penetrance
P.....	238	6	2.5	...	...	..
F ₁	340	14	4.0	79	9	10
F ₂	268	1	0.4	115	22	16
F ₃	216	1	0.5	sterile		
F ₄	135	..	...			

TABLE 25
SELECTION IN A VERY LOW PENETRANCE tet LINE

Generation	Matings + × +			Matings tet × tet			Remarks
	+	tet	penetr.	+	tet	penetr.	
P.....	697	1	0.1	...	..	...	5 broods
F ₁	1,407	11	0.7	...	..	...	8 broods, one with 10 tet offspring from P brood with one tet
F ₂	804	..	...	286	1	0.4	5 broods, 4 from same F ₁
F ₃	511	..	...	...	..	...	3 broods
Total...	3,705	12	0.3	...	..	...	

B. PODOPTERA G

The establishment of a probably homozygous pod-G line by selection was reported above (table 1, p. 70). In the whole selection experiment, involving almost 200,000 flies, the penetrance was only 0.26 per cent. If only the generations (indicated in the table) are considered in which pod may be supposed to have been extracted, the following values for penetrance were obtained in the different lines:

Line	1	2	3	4	5	6	7	8	9	10	11
Pen.	0.1,	0.17,	0.21,	0.25,	0.4,	0.5,	0.55,	1.2,	1.4,	1.5,	1.8

The incidence is so small that a further analysis by selection seemed hardly feasible. Thus, it cannot be stated whether these lines differed in regard to the number of pod factors, or in specific modifiers, or in nonallelic pod factors of

different potency (measured by their penetrance). Therefore, the line with highest penetrance—marked by $\text{In}(1)y^{px\ bl}$ and $\text{In}(1)w$ —was isolated and bred as the pod-G line. It showed a constant small penetrance whether breeding was done from normal or from pod flies. Table 26 contains some of the pertinent data.

Later, a control was made in connection with temperature experiments (see below) with the result of 0.4 per cent penetrance, breeding from normals (692 individuals). As the line did not seem to be good material for penetrance selection, an isogenic stock was made from the same line starting with a

TABLE 26
PENETRANCE IN EXTRACTED pod-G, ONE-PAIR BROTHER-SISTER
BROODS

Matings	Flies +	pod	Percentage of penetrance
+ × +.....	3,073	20	0.64
+ × pod.....	1,108	10	0.87
pod × pod.....	1,197	10	0.83

TABLE 27
PENETRANCE IN FIVE GENERATIONS OF ISOGENIC pod-G

Matings	Broods	Number		Percentage of penetrance	Lowest penetrance	Highest penetrance
		+	pod			
+ × +.....	3	555	9	1.6	...	3.1
+ × pod.....	13	1,667	64	3.7	1.1	10.0
pod × pod.....	25	3,077	103	3.2	...	9.6

phenotypically pod individual. Since the isogenic stock is practically homozygous (except for the fourth, and possibly part of the third, chromosome, which is not completely protected from crossing over), the pod factor or factors should be almost homozygous. The penetrance of pod in five generations of tests of this isogenic line are shown in table 27.

Penetrance in the isogenic stock is much higher than in the extracted stock but varies considerably in broods of about equal size from 0–10 per cent. The detailed results of 4 generations of selection for pod and + phenotypes in this line were not clearly negative as they should be in a completely isogenic line. Therefore, a large-scale selection experiment was carried out with the isogenic line.

Unfortunately, such experiments cannot be conducted as a strict selection experiment. With as low a penetrance as was encountered, brother-sister matings with pod-type individuals from single-pair matings are frequently impossible. Therefore, only rarely can a strict-selection line be continued through many generations. Actually, only 6 such lines could be maintained for 4 generations or more. The results are presented in table 28. In lines 1 and 2

TABLE 28

SELECTION OF pod PHENOTYPES IN SINGLE-PAIR BROTHER-SISTER MATINGS OF ISOGENIC pod-G WITH STRICT INBREEDING OF pod PHENOTYPE

No.		F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	M
1	Broods.....	1	1	1	1	1	1	3	1	
	Individuals.....	156	70	64	182	102	94	444	161	
	Percentage pod penetr.....	2.0	4.3	4.7	2.2	2.0	6.4	6.3	5.6	4.1
2	Broods.....	1	1	1	1	1	1			
	Individuals.....	122	84	98	244	204	122			
	Percentage penetr.....	1.6	7.2	5.1	5.7	5.4	6.5			5.5
3	Broods.....	1	1	1	1	1				
	Individuals.....	133	163	190	143	149				
	Percentage penetr.....	6.0	6.1	5.3	4.2	6.0				5.5
4	Broods.....	1	1	1	1	1				
	Individuals.....	105	205	155	228	81				
	Percentage penetr.....	6.7	4.9	6.5	3.9	1.2				4.5
5	Broods.....	1	1	1	1	3				
	Individuals.....	197	65	149	180	328				
	Percentage pod penetr.....	3.6	3.0	8.8	2.6	1.5				3.3
6	Broods.....	1	1	1	1					
	Individuals.....	122	84	98	158					
	Percentage penetr.....	1.6	7.2	5.1	3.2					4.1

TABLE 29

MEAN PENETRANCE IN STRICTLY INBRED BROODS OF pod-G PHENOTYPES (TABLE 28) COMPARED WITH PENETRANCE IN THE SA NBRED LINE
IN ALL CLASSES (pod X pod and pod X + INCLUDING COUSIN MATINGS)

Line	No. in table 28	Main line pod X pod				Main line pod X +				Inbred broods from table 28	
		Brother-sister		Cousin		Brother-sister		Cousin		Indiv.	M penetr.
		Indiv.	Penetr.	Indiv.	Penetr.	Indiv.	Penetr.	Indiv.	Penetr.		
14370.....	1	7,552	4.2	1,805	2.9	5,185	3.2	979	4.3	1,273	4.1
14370.....	2		...		...		...	...	...	874	5.5
14370.....	5		...		...		...	...	...	928	3.3
14370.....	6		...		...		...	...	...	462	4.1
14372.....	3	10,094	3.2	1,815	5.3	5,837	3.1	426	4.5	778	5.5
14349.....	4	4,008	3.3	329	5.2	3,379	3.5	...	...	774	4.5

it appears that after a short selection a high-penetrance line was selected. Line 3 looks as if it had been selected from the beginning for high penetrance. But line 4 shows a more or less decreasing penetrance, and lines 5 and 6 are completely irregular. In order to draw conclusions from them these data must be compared with data for broods of the same line less strictly selected for pod phenotypes. Table 29 contains the data arranged according to lines and types of mating. Within each line we have, in the order of decreasing inbreeding and selection (1) the strictly selected lines (2) brother-sister inbreeding (3) cousin breeding. In lines 2 and 3 the records for (a) pod $\times$ pod and (b) pod $\times$ + matings were kept separate. If the isogenic stock can still be selected, normal individuals should have more chance to be of a minus constitution in regard

TABLE 30
COMPARISON OF ALL SELECTION GROUPS IN pod-G
(Pod $\times$ pod includes the strict selection. The table is arranged according to mating of phenotypes pod $\times$ pod or pod $\times$ +, not distinguishing between brother-sister and cousin matings.)

	pod $\times$ pod	pod $\times$ +	pod $\times$ pod strict selection
Total number.....	37,193	28,846	5,089
Number pod.....	1,360	1,018	228
Penetrance.....	3.7	3.5	4.5

to pod factors, although the chance is small in view of the low penetrance even in the best case. Thus, with effective selection for pod factors the percentage penetrance should be $1 > 2a > 2b > 3a > 3b$. The actual values are in this order:

	1	2a	2b	3a	3b
For line 14370:	4.1	4.2	3.2	2.9	4.3
For line 14372:	5.5	3.2	3.1	5.3	4.5
For line 14349:	4.5	3.3	3.5	5.2	

Thus, selection does not clearly increase penetrance. Comparison of the average penetrance of the strictly selected lines with all the other less strictly selected ones shows 4.3 per cent penetrance for the strictly selected ones among 5,089 individuals, and 3.5 per cent among 41,409 individuals for the less selected group. The χ^2 of the difference is 7.24 $p < 0.01$, i.e., significant.

In table 30 all the matings pod $\times$ pod are compared with all matings pod $\times$ + (including lines not contained in table 29), and these again are compared with the strict-selection group in table 28.

The results are finally presented in a graph (fig. 23) indicating the course of selection in the two largest individual lines, each starting with one pair after 4 generations of brother-sister inbreeding. In this graph a distinction is made between pod $\times$ pod and pod $\times$ + matings. All matings are brother-sister matings, and the parents—also brother-sister—may have been either pod $\times$ pod or pod $\times$ + phenotypes. The curves indicate the same trend as noticed

in the tables. As a whole, the pod $\times$ pod curves keep above the pod $\times$ + curves, and there is a very slight tendency toward positive selection effects. Only the curve for line 14,273 with pod $\times$ + matings is completely irregular. It may be concluded that the slight increase in penetrance which can still be obtained by selection in this isogenic line is probably based upon the incomplete homozygosity in the fourth and part of the third chromosome, which contain either low podoptera factors or positive modifiers. There is no indication of frequent mutation in isogenic pod-G.

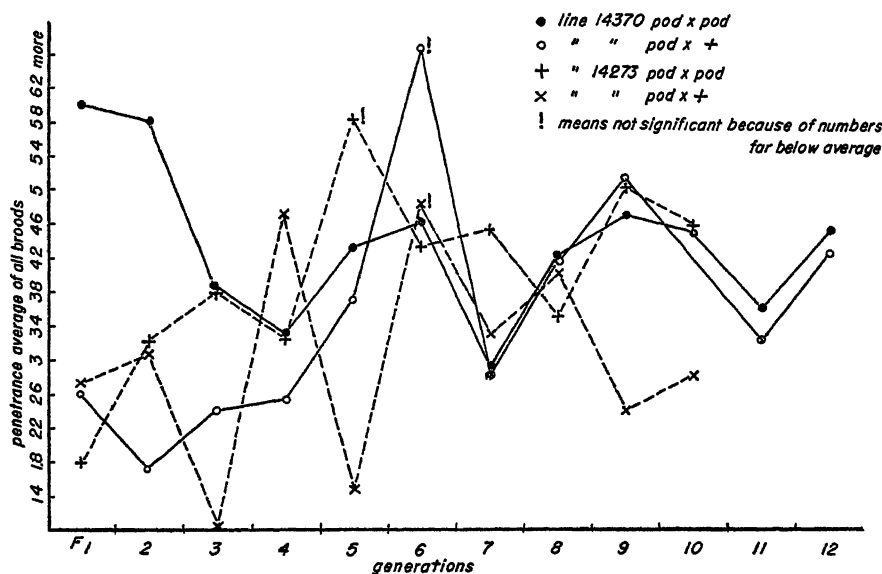


Fig. 23. Results of selection of podoptera G.

C. PODOPTERA H

The pod-H line was derived from a line of the Florida Iso '39 strain which showed a relatively high incidence of podoptera, i.e., 1.18 per cent among 340 flies (inbreeding by single-pair matings of phenotypically normal flies for 6 generations). In F_7 the incidence was 4.1 per cent among 611 flies (5 matings). One mating, no. 704c, had 5.71 per cent podoptera among 175 flies. The high incidence of podoptera continued for three more generations: $F_2 = 2.86$ per cent, $F_3 = 7.44$ per cent; $F_4 = 7.11$ per cent (table 31). This line was therefore chosen for analysis and was designated pod-H. Because of its relatively high penetrance and a number of specific peculiarities, pod-H was used for an elaborate selection experiment.

a. General

The increase in incidence from 1.18 per cent to 5.71 per cent is statistically significant ($\chi^2 = 5.27$). Therefore it appeared likely that either segregating podoptera factors had become homozygous, or that a new mutant had appeared and was selected by chance. If several segregating factors had been involved,

the incidence of podoptera in closely related broods should be significantly different from that in the broods which are separated by several generations. The data in table 31 show this to be the case. Direct descendants of the flies from the mating number 704c (5.71 per cent pod) had an incidence of 6.33 per cent pod among 633 flies, whereas matings of brother and sister had only 2.81 per cent among 533 flies, first cousins 2.71 per cent among 517 flies, and second cousins 2.82 per cent among 319 flies. χ^2 values are significant in each case ($\chi^2 = 7.03, 7.19$, and 4.67). These data may be interpreted:

(1) The significant difference in penetrance between sibling broods and broods of matings of distantly related flies indicated that a strong pod factor

TABLE 31

INCIDENCE OF PODOPTERA FLIES IN THE FIRST FOUR GENERATIONS OF THE pod-H LINE AND IN MATINGS NOT IN THE DIRECT LINE OF DESCENT

Generation	Direct pod-H line			Brother-sister matings not in line			1st cousins			2d cousins		
	Total flies	pod	Per-centage pod	Total flies	pod	Per-centage pod	Total flies	pod	Per-centage pod	Total flies	pod	Per-centage pod
F ₁ ...	175	10	5.71	315	10	3.11	239	3	1.25	...	..	
F ₂ ...	70	2	2.86	149	2	2.68				...	..	
F ₃ ...	121	9	7.44	69	1	1.45	236	9	3.18	...	..	
F ₄ ...	267	19	7.11	...	..		42	2	4.90	319	9	2.82
	633	40	6.33	533	13	2.81	517	14	2.71	319	9	2.82

(giving a higher incidence of pod) was present in the flies of the selected line but either was not present or was segregating in the other lines.

(2) The relatively high incidence of pod among flies not in the direct line suggests that the penetrance of pod-H is dependent on multiple factors, and that some of these factors may have been homozygous, because there is not a significant difference in penetrance among the three groups (columns 2, 3, and 4).

(3) Finally, the penetrance of pod per generation increased; therefore, there must be other factors which were still segregating at the end of the fourth generation.

b. Selection of podoptera H in special lines

a. A line with high incidence of podoptera

The extreme variability in penetrance and expressivity of the podoptera phenotypes in the pod-H line even after it had become homozygous for at least one factor suggested the possibility that some factors of the complex may have been heterozygous and segregating, and that the expression of the podoptera effect in the progeny depended upon the genotype of the parents. To test this hypothesis five closely related lines (all with high penetrance and all derived from the cross no. 704c) which differed only in parental phenotypes and types

of flies mated were inbred for a number of generations. The parental phenotypes were:

Line no. 1073 podoptera type II ♀ × normal ♂

Line no. 1080 gynandromorph ♀ × normal ♂

Line no. 1081 podoptera type II ♀ × normal ♂

Line no. 1083 podoptera type II ♀ × normal ♂

Line no. 1084 podoptera type II, gynandromorph ♀ × normal ♂

Lines 1073, 1081, and 1083 were selected for podoptera, line 1080 for gynandromorphs, and line 1084 for both. Single-pair matings were made of the types designated by the line. However, as the gynandromorphs and podoptera in particular were not very viable and had low fertility, matings of phenotypically normal flies (brothers and sisters to the abnormal flies) were made simultaneously. If the first mating proved to be sterile, the lines were maintained by the "normal" matings. Likewise, if no podoptera phenotypes appeared among the offspring, the line was continued by matings of phenotypically normal flies. Consequently, in every line the highest percentage of crosses were matings of normal flies. The other cross types varied from line to line.

The lines were inbred for 17 or 18 generations, except line 1083, which was lost in the fifteenth generation (table 32). At the end of this time, 599 podoptera flies among 17,249 flies, or 3.47 per cent, distributed in the 6 classes, had been observed (table 33). The highest incidence of podoptera per mating was 20.9 per cent. Numerous matings had no podoptera, but as the average number of flies per bottle was 56, this was to be expected. It was possible to determine whether extreme deviations are significant by finding the average incidence after combining all the progeny of a single generation in each line. On this basis the incidence of podoptera varied from 0.00 to 8.96 per cent, with a mean of 3.25 per cent. On the assumption that the deviations from the mean are due to random sampling of a normal distribution, the 64 samples giving $\chi^2 = 7.105$ (9 df), which is not significant.

However, the variance between the lines gave an F value of 7.33 (4 + 59 df), which is highly significant.² Although the variance in incidence of podoptera is not significant if all the lines are combined, there is a significant difference between the mean incidence of podoptera in the 5 inbred pod-H lines.

These data may be interpreted in several ways: (1) Mating of specific phenotypes selected enhancing modifiers or multiple factors of different potency for that phenotype. (2) Specific inhibiting factors were selected in certain lines. (3) The genotype of all individuals is the same, but the expression of the effect is based upon environmental action. If the difference in penetrance between the lines depends upon selection and fixation of multiple factors, the lines should show an increase in incidence of podoptera concurrent with inbreeding, especially the lines that had a higher incidence of podoptera. Actually, lines 1081 and 1084 showed an increase, line 1083 a slight decrease (fig. 24). All the lines combined had a slight but statistically insignificant increase in penetrance. It seems evident that selection for podoptera increases the penetrance of the podoptera effect.

² F is the variance ratio (Snedecor, 1946).

TABLE 32
INCIDENCE OF PODOPTERA AND GYNANDROMORPHS PER GENERATION IN THE INBRED pod-H LINES (EXPERIMENT I)

Generation	Line 1073			Line 1080			Line 1081			Line 1083			Line 1084			2		
	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn
1		..	..		..	..		..	..		..	..		..	..	490	20	0
2		..	..		..	..		..	..		..	..		..	..	188	6	1
3		..	..		..	..		..	..		..	..		..	..	426	19	1
4		..	..		..	..		..	..		..	..		..	..	628	30	2
5		..	..		..	..		..	..		..	..		..	..	167	1	3
6	4	..	..	31	..	..	46	1	..	40	..	2	46	..	1	646	19	4
7	39	2	..	168	5	2	123	7	1	210	3	..	106	2	1	1,050	13	3
8	42	1	1	98	1	..	150	4	..	553	5	1	207	2	1	603	19	5
9	149	7	..	51	..	2	144	4	1	150	3	..	109	5	2	1,039	27	5
10	211	12	1	104	3	..	87	3	..	199	1	1	438	8	3	1,388	49	4
11	117	6	..	77	4	..	173	7	..	243	4	..	778	28	4	1,391	42	4
12	406	26	..	136	4	1	283	5	..	239	5	2	589	18	1	2,071	70	3
13	434	25	..	159	8	1	145	13	1	252	2	..	1,109	21	1	2,023	72	4
14	191	10	..	247	6	1	279	12	..	122	..	..	911	29	3	1,478	55	8
15	288	5	2	820	3	4	161	12	2	166	1	1	610	29	1	1,361	46	12
16	102	5	..	379	16	8	147	10	..	..	..	..	547	15	2	820	43	2
17	148	5	..	190	6	..	67	2	..	..	..	..	461	30	2	951	45	7
18	...	..	..	285	6	7	73	5	..	..	..	..	445	29	..	529	23	4
	...	..	..	136	3	3	...	..	..	..	..	..	393	20	1			
	2,275	114	4	2,381	65	29	1,878	85	5	2,174	24	7	6,809	236	23	17,249	599	72

TABLE 33
PODOPTERA TYPES IN INBRED pod-H LINES

[illegible]

The low-penetrance characteristic of pod-H made it impossible to conduct a direct selection experiment; therefore, the effectiveness of selection can be determined only indirectly. Comparison of the incidence among the offspring of different matings, i.e., normal $\times$ normal, podoptera $\times$ podoptera, gynandromorph $\times$ normal, and "etched abdomen" $\times$ normal, determines whether selection is directly effective in the F_1 generation. By the analysis of variance test, the percentage of podoptera in these 4 matings give an F value of 0.634 (13

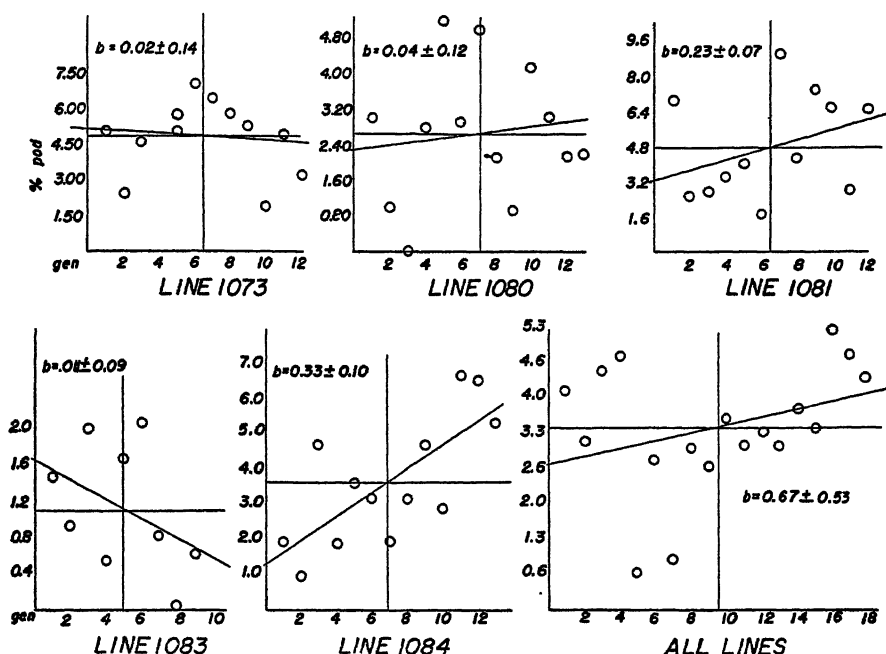


Fig. 24. Pod-H. Linear regression test showing the increase of penetrance of podoptera in five inbred lines.

and 16 df) which does not constitute a significant deviation. Therefore, the matings of podoptera, gynandromorphs and "etched abdomen" do not have more podoptera flies among their F_1 offspring than the matings in which the parents were phenotypically normal. It seems probable that the phenotype is not necessarily an expression of the genotype, e.g., the extreme phenotype having more, or more potent, pod factors.

If the significant difference between lines is not due to selection of podoptera-enhancing factors, it may be due to selection of inhibiting factors. In the first four generations crosses leading to lines 1073 and 1083 had a low incidence of flies per mating, an indication that viability and (or) sterility factors were also present. It had already been observed that a higher incidence of podoptera was frequently found in bottles with the lower incidence of flies (table 34). Therefore, in line 1083, parents for the next generations were always selected from bottles with the highest incidence of podoptera and lowest number of flies. This line not only had a low incidence of podoptera, but the penetrance

showed a slight decrease on inbreeding, and the whole line was lost by the fifteenth generation, probably because of the accumulation of inviability and sterility factors. The podoptera flies are not very viable and are frequently sterile even in lines which have no lethals (see below: Isogenic lines). It is not unreasonable to suppose that podoptera flies are unable to survive when they have other deleterious mutations.

Line 1073, which was separated from the other lines in the F_2 generation, also showed a high penetrance of podoptera combined with a low incidence of flies (table 34). When the selection experiments were made, line 1073 was

TABLE 34
INCIDENCE OF FLIES AND PODOPTERA PER BOTTLE IN LINE 1073 COMPARED
WITH THE OTHER LINES OF pod-H

	Matings leading to line 1073				Other lines			
	Matings	Total flies	pod	Percentage	Matings	Total flies	pod	Percentage
Mating 704c....	..	..	..		1	175	10	5.71
F_2	1	15	..		1	55	2	3.64
F_3	1	112	9	8.04	1	9	..	
F_4	1	24	5	20.83	2	243	14	5.76
Σ	3	151	14	9.27	5	482	26	5.40
Average no. of flies per bottle	50				96			

maintained primarily by matings of flies from bottles with the highest incidence of flies to select against the deleterious sterility and viability factors. This line showed a slight (but insignificant) decrease in the incidence of podoptera. These data suggest that the podoptera factor or factors are detrimental, or that semilethality or semisterility mutants are closely linked to podoptera factors.

In spite of the fact that the incidence of podoptera is not significantly different in the progeny of flies with the podoptera phenotype, the fact remains that some lines do have a significantly higher incidence, and that that difference may be dependent, first, upon the parental phenotype, and second, upon the length of time of inbreeding of specific phenotypes. As stated before, to insure continuity of a line whenever a podoptera or gynandromorph mating was made, a mating was also made of phenotypically normal brothers and sisters. Of 9 pairs in which one mating was gynandromorph $\times$ normal and the other normal, the gynandromorph matings had an average incidence of 3.52 per cent podoptera, the normal matings only 2.64 per cent. Of the 6 pairs of podoptera $\times$ normal and the normal $\times$ normal matings, the podoptera matings had 5.30 per cent and the normal matings 3.35 per cent. Although the differences are not statistically significant, the data point to the possibility that siblings may differ in their genotypes. If this assumption is true, continuous inbreeding of

gynandromorphs or podoptera should increase the penetrance of podoptera (as was shown to be true for tet). However, in the case of pod-H the increase in penetrance does not occur even in the second generation (table 35); in fact, the decrease in penetrance of the podoptera crosses from 4.0 per cent to 1.9 per cent is statistically significant. Accompanying this decrease in incidence is a decrease in the total number of flies per bottle. Therefore, inbreeding podoptera not only decreases the incidence of podoptera offspring but de-

TABLE 35
INCIDENCE OF PODOPTERA H IN TWO GENERATIONS OF INBREEDING

	Total crosses	1st generation			2d generation			x ²
		Total flies	pod	Percentage	Total flies	pod	Percentage	
Podoptera crosses.....	19	1,158	46	4.0	571	11	1.9	4.83*
Gynandromorph crosses.....	5	171	6	3.5	316	9	2.8	0.17

* Following the method used by Snedecor, we use an asterisk, in this and subsequent calculations, to denote the probability between the 5 per cent and 1 per cent levels.

TABLE 36
INCIDENCE OF PODOPTERA H IN FIVE GENERATIONS BREEDING FROM pod

	Matings	Sterile matings	Total flies	pod	Percentage of pod
F ₁	2	..	134	12	8.95
F ₂	3	..	146	23	15.75
F ₃	5	1	230	10	4.35
F ₄	3	1	73	6	8.22
F ₅	2	1	42	..	

creases the viability and (or) fertility. The gynandromorph crosses likewise showed a decreased number of podoptera offspring in the F₂ generation, but in this case the decrease is not significant.

Inbreeding of podoptera phenotypes was possible for several generations in two cases (one in line 1073 and one in 1081). The combined data (table 36), show that inbreeding may increase the penetrance of podoptera for several generations, but that it is eventually reduced. Inbreeding also resulted in poor viability and increased sterility. Therefore, it seems unlikely that the penetrance of pod-H can be increased as the result of inbreeding and selection of specific phenotypes, especially podoptera, because nonviability and sterility also increase, resulting in a decrease in the incidence of podoptera. Selection of podoptera in the pod-H line is actually a negative selection for penetrance.

In summary, the following generalizations seem warranted:

1. Two lines which had a significantly higher incidence of podoptera also showed a linear increase in penetrance on inbreeding.

2. Inbreeding specific phenotypes, namely, podoptera and gynandromorphs, may give a higher incidence of podoptera, especially in the first generation, but the incidence decreases in subsequent generations.

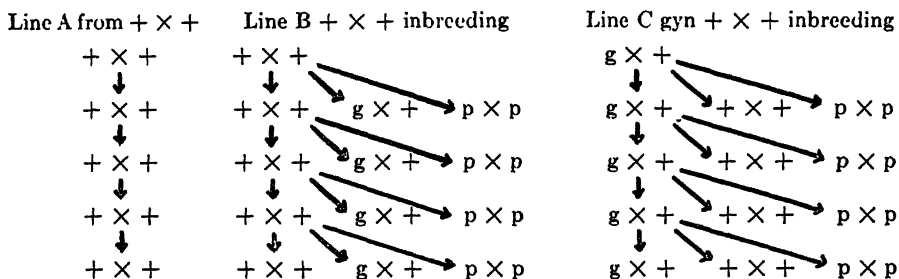
3. Associated with, or a part of, the podoptera effect are sterility and inviability effects, which increase proportionally with the amount of inbreeding.

β. A line of high incidence of gynandromorphs

In order to establish the exact relationship existing between parental phenotypes and the incidence of podoptera in the progeny, and to determine the effectiveness of inbreeding, a second experiment was designed so that the variation within lines could be tested simultaneously with variation between

Pod-H: PEDIGREE OF MATINGS IN SELECTION EXPERIMENT II

(Line A from unselected mass matings; lines B and C from mass matings of gyn and pod)



lines. Two strains were established in the following way: A mass mating was made with the progeny of mating 1235 (line 1084) which had an incidence of 4.58 per cent podoptera and 1.83 per cent gynandromorphs. This line was carried by mass matings without selection for 10 generations. At this time a mass mating was made of podoptera and gynandromorphs. This line was selected for these two phenotypes in succeeding generations. At the end of 20 generations for the unselected line, and 10 for the selected line, 5 single-pair matings of phenotypically normal flies were made from each line, and 5 single-pair matings of gynandromorphs (gynandromorph × normal male) from the second line. Three podoptera matings from the second line were made at the same time, but all were sterile. In later generations, likewise, although all the podoptera flies were mated, it was impossible to maintain a podoptera line for even 2 generations.

The two groups of normal matings, designated "A" and "B," were maintained for 5 generations by 5 single-pair matings of phenotypically normal flies selected from one bottle (pedigree above). In group B the podoptera and gynandromorph flies were also bred, but no matings were made from the progeny. Line C (the gynandromorph line) was continued for 5 generations by gynandromorph matings. Five matings of phenotypically normal flies from the gynandromorph cross with the highest incidence of podoptera were also made in each generation. Likewise, all the podoptera flies were bred in each generation.

The accompanying tables show the available statistics for the relationship between parental phenotypes and the incidence of podoptera. Table 37 compares the incidences of podoptera and gynandromorphs in the inbred lines of this experiment (II) to those of the first selection experiment (I, table 32).

The incidence of podoptera is significantly lower in all but two sublines: line (b), inbreeding normals from A, and line (f), inbreeding normals from B. These results may be interpreted to mean that, as the result of mass matings for a number of generations, the podoptera factor had lost some of its expressivity, or that the as yet undetermined modifiers or minor pod factors had been eliminated.

TABLE 37

COMPARISON OF INCIDENCE OF PODOPTERA H IN LINES HAVING A HIGH INCIDENCE OF pod (I) WITH LINES HAVING A HIGH INCIDENCE OF GYNANDROMORPHS (II)

Selection lines	Total flies	pod	χ^2 between total pod in experiments I and II
Experiment I. High-pod lines—total.....	17,249	599	
(a) Experiment II. High-gyn. lines—total.....	9,776	255	13.95**
(b) Line A—inbreeding normals.....	1,016	30	1.60
(c) Lines B and C—high gynandromorphs....	8,760	225	16.40**
(d) Line B—total.....	3,207	85	6.43**
(e) Line C—total.....	5,553	140	10.38**
(f) Line B + $\times$ + crosses.....	1,592	40	2.62
(g) Line C + $\times$ + crosses.....	2,233	49	8.25**
(h) Lines B and C + $\times$ + crosses.....	3,825	89	11.91**

** The double asterisk indicates that χ^2 is significant. The incidence of pod in line II is lower than in line I.

However, two lines of evidence suggest that no loss of podoptera factors had occurred. As shown before, the parental types may determine to an extent the expression of the podoptera action among their offspring. Experiment II differs from experiment I in respect to the concentration of parental types. Only two lines in experiment I had a significant number of gynandromorph matings; in experiment II, 3,879 flies, or one-third of the total number of flies, were offspring of gynandromorphs. The data presented in table 38 suggest that the incidence of podoptera may be decreased in gynandromorph crosses, and that the incidence of gynandromorphs may be increased. This is further substantiated by the fact that in both experiments I and II the incidence of podoptera shows a negative correlation with the incidence of gynandromorphs, i.e., if the incidence of gynandromorphs is increased, podoptera numbers are reduced (p. 105). Therefore, it was to be expected that the incidence of podoptera might be less in this experiment, both because of breeding from gynandromorphs and the high incidence of gynandromorphs among the progeny of all crosses.

Second, the incidence of podoptera in the two lines—designated (b) and (f) in table 37—which were maintained by inbreeding phenotypically normal flies did not differ significantly from the incidence of podoptera in experiment I,

TABLE 38

Pod-H: INCIDENCE OF PODOPTERA AND GYNANDROMORPHS PER GENERATION IN EXPERIMENT II

Generation	Line A			+ X + crosses						gyn X + crosses						pod crosses					
				B			C			B			C			B			C		
	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn	Total flies	pod	gyn
1.....	82	3	6	420	11	7	420	11	7	389	9	7	389	9	7		..	..		..	..
2.....	291	8	14	317	9	6	459	4	13	195	8	7	293	12	10	20	1	..	189	12	1
3.....	251	5	10	357	12	7		..	..	288	9	2	408	12	16	62	3	1	116	2	2
4.....	189	6	5	298	8	10	844	14	21	33	2	..	910	20	9	121	3	5	330	9	3
5.....	203	8	4	200	..	5	510	20	8	355	4	8	619	14	15	152	6	5	66	1	1
Σ.....	1,016	30	39	1,592	40	35	2,233	49	49	1,260	32	24	2,619	67	67	355	13	11	701	24	7

Line A: Inbred phenotypically normal flies—unselected.

Line B: Inbred phenotypically normal flies and outbred gynandromorphs and podoptera—selected.

Line C: Inbred phenotypically gynandromorphs and outbred normal and podoptera—selected.

Selected: Phenotypically gynandromorph and podoptera bred by mass matings for 5 generations, before experiment II.

Unselected: Phenotypically normal flies bred by mass matings for 10 generations, before experiment II.

which was made up primarily of matings of phenotypically normal flies. This points to the probability that the genetic constitution of the flies of this experiment was identical with that of the previous experiment, and therefore that the fluctuations in incidence are due to random sampling rather than to the loss of modifying factors.

The design of experiment II made it possible to compare the incidence of podoptera by the *t* test using the pairing design method. The results (table 39) show that: (1) No significant change in the incidence of podoptera resulted from selection vs. nonselection in the mass matings previous to this experiment.

TABLE 39

COMPARISON OF INCIDENCE OF pod-H AS THE RESULT OF INBREEDING AND OUTBREEDING:
A, INBRED—FROM NONSELECTED; B, INBRED—FROM SELECTED LINES; C, INBRED
gyn FROM SELECTED LINE

	$\bar{x}$	Sx	df	P
+ to + A to B.....	0.70	1 59	4	>0.5
+ to + B to C.....	0.21	0.44	3	>0.5
g to g B to C.....	0.86	0.84	4	0.4-0.5
p to p B to C.....	0.99	1 07	3	0.4-0.5
+ to g B.....	1.21	0.61	4	0.2-0.1
+ to p B.....	1.81	0.87	3	0.2-0.1
g to p B.....	0.27	1.35	3	>0.5
+ to g C.....	0.44	1.02	3	>0.5
+ to p C.....	1.38	2 28	2	>0.5
g to p C.....	0.22	0.78	3	>0.5

P=pod, g=gynandromorph.

(2) Although there may have been an apparent decrease in incidence of podoptera concurrently with an increase in incidence of gynandromorphs, as a result of selection, the difference is not significant. (3) The parent offspring correlation is not statistically significant. (4) Although the penetrance cannot be increased significantly by inbreeding, the incidence of podoptera is likely to be in the upper range of variability in the podoptera crosses, and in the lower in the gynandromorph crosses.

Inbreeding gynandromorphs increased the incidence of gynandromorphs and reduced the incidence of podoptera; but this reduction was not significant, i.e., the range of deviations did not fluctuate beyond the limits of the standard deviation. But the most important information derived from these data is that the factor determining the expression of gynandromorphism is probably identical with that determining podoptera.

γ. Isogenic lines of podoptera H

Four isogenic lines were made by the Muller method (Dis 6) in the course of the experiment from flies of various phenotypes. Because of sterility and inviability factors inherent in the Fla Iso '39 stock, only one line (1235) was actually isogenic in the fourth generation. Line 1379 became isogenic in the

fifth generation, but in lines 1449 and 2744 either the podoptera third chromosome was always balanced with dominant markers, or the nonmarked flies were sterile.

Each isogenic line was maintained for several generations by inbreeding many single-pair matings in a manner similar to that described for the inbred pod-H lines, i.e., using podoptera phenotypes when possible, and phenotypically normal flies, otherwise. The incidence of podoptera per generation for each of the four lines is given in table 40. The two pertinent facts brought out by these data are: (1) The incidence of podoptera was relatively low in the

TABLE 40
INCIDENCE OF PODOPTERA IN INBRED pod-H ISOGENIC LINES

Parent	Line 1235		Line 1379		Line 1449		Line 2744	
	+ ♂		Hd ♂		Hd gyn ♀		+ ♂	
Marker	None		None		Sb		H	
	Total flies	Percent- age pod	Total flies	Percent- age pod	Total flies	Percent- age pod	Total flies	Percent- age pod
F ₄	124	0.80	...		58		182	2.74
F ₅	180	0.55	515	1.35	193	3.10	110	1.81
F ₆	340	0.88	586	1.53	458	6.10	192	4.15
F ₇	488	1.43	220	2.72	409	3.1	41	4.87
F ₈	9	11.11	15 matings sterile		205	5.3	23	
F ₉	sterile				6 matings sterile			

first generation after the line had become isogenic, but increased very rapidly on inbreeding. (2) The appearance of sterility effects was very sudden; in the eighth or ninth generation all but one of the lines was lost because every mating was sterile.

One isogenic line (1235) was also maintained by mass matings as well as single-pair matings. After a number of generations, it was again tested for pod penetrance. The line was then inbred for four generations by making four single-pair matings of phenotypically normal flies from the brood which had the highest incidence of podoptera. The results are summarized in table 41. Essentially the same results were observed: Inbreeding increased the incidence of podoptera rapidly, but the number of flies per bottle decreased with as great rapidity, with the result that, finally, the F₅ matings were sterile.

An isogenic fly is actually only isogenic for the first, second, and the greater part of the third chromosome (the H chromosome permitting crossing over), and the fourth is segregating. Thus, even in a line that is isogenic in the fourth generation, further selection is possible. If heterozygous podoptera factors were present, they would eventually become homozygous or be eliminated. On this assumption the increase in incidence of the pod-H isogenic line could be explained as selection and fixation of heterozygous podoptera factors. But it is difficult to believe that factors in these two rather small regions are more

powerful than all the rest of the podoptera factors in the other chromosomes, and it would be necessary to make this assumption, for the incidence increased three- to four-fold in each line, even in line 1235, in which there was a deliberate negative selection for podoptera. The alternative hypothesis is that podoptera-enhancing mutants appear after the isogenic line has been established. If this is the case, the following assumptions would have to be made: (1) podoptera-enhancing mutants appear frequently and quickly become fixed by selection, or (2) the rapidly mutating enhancers are dominants which have a marked effect, but probably do not become homozygous, hence the fluctuating penetrance, (3) the podoptera mutants are simultaneously semilethals or

TABLE 41
INCIDENCE OF PODOPTERA IN INBREEDING PHENOTYPICALLY NORMAL FLIES FROM
pod-H ISOGENIC LINE 1235

Generation	Total number of matings	Total flies	Total pod	Percentage of pod
F ₁	4	347	3	0.84
F ₂	4	218	11	5.02
F ₃	4	178	7	3.92
F ₄	4	32	3	9.33
F ₅	2	...	..	
Total.....	..	775	24	

sterility genes, or (4) the mutation and accumulation of sterility or semilethal genes in addition to the pod-enhancers, is very rapid, or (5) a dominant sterility factor appears suddenly in the eighth or ninth generation of every line.

It is possible that both selection of factors already present in the nonisogenic chromosomes and selection of newly mutated enhancers would account for the rapid increase in incidence of podoptera in the pod-H isogenic lines from the fourth to the seventh generation. The genetic analysis below will throw more light upon the factors involved.

D. PODOPTERA K

At the onset of a selection experiment, i.e., after the pod-K stock had been bred by mass cultures from podoptera phenotypes for several generations, 4 types of matings, pod × pod, pod × ro-l, no-l, ro no-l × pod, and ro-l no-l × ro-l no-l were made. A summary of the penetrance of podoptera in the 4 groups of matings is presented in table 42. There is much variation among counts of the individual cultures which make up the total. Some of the high variation observed is probably due to the fact that we are not comparing offspring of single-pair matings, since three phenotypically podoptera flies and two ro-l, no-l flies had to be used in the matings because of the low fertility of pod-K flies, especially of the phenotypically podoptera individuals. The variation between cultures of each group shows also that the podoptera stock was not homozygous for all pod factors at the onset of the selection experiment.

The penetrance of podoptera in all 4 groups of matings is rather similar. The value obtained for ro-l no-l $\times$ pod is higher than the others. The sample is, however, rather small, therefore the higher penetrance value found is almost certainly a chance result. In all cultures, males and females appeared in about equal numbers. Bilateral types occurred with variable frequency in a number of cultures.

Three pairs of phenotypically podoptera flies were bred from one culture which yielded 55.8 per cent podoptera, and selection for podoptera was con-

TABLE 42
PENETRANCE OF pod-K IN DIFFERENT TYPES OF MATINGS

Phenotypes mated	Total	Percentage of pod
pod $\times$ pod.....	316	12.8
pod $\times$ ro-l no-l.....	888	10.1
ro-l no-l $\times$ pod.....	248	20.6
ro-l no-l $\times$ ro-l no-l.....	1,154	13.8
Σ	2,606	13.0

TABLE 43
SELECTION OF pod PHENOTYPES IN pod-K (EXPERIMENT I)

Generation	Total	Percentage of pod	Expressivity
F ₁	43	55.8	All pod are class VI or bilateral
F ₂	No count	Good penetrance of pod	
F ₃	412	53.1	Mostly class VI and bilateral
F ₄	218	63.7	

tinued for 4 generations. The results are shown in table 43. Since high penetrance of podoptera was maintained in all the 4 generations, but no further increase in penetrance due to inbreeding was observed, the P individuals must have carried a nearly homozygous and rather viable combination of pod factors.

After the original stock had been maintained for many generations by mass selection of pod $\times$ pod, another selection experiment was made. The results are summarized in table 44. The penetrance varied between 40 and 60 per cent, with no apparent increase due to selection, thus being in complete agreement with the earlier results.

E. PODOPTERA M(3)124

Two sets of selection experiments were made with this stock—one shortly after it had been established, and a second one after it had been maintained for a long time by mass selection of podoptera.

Results of the matings pod $\times$ pod, pod $\times$ + and + $\times$ + which served as the starting point for the first selection experiment are shown in table 45. In the small sample the incidence of pod in the mating pod $\times$ pod is higher than in the other matings. Pod and normal phenotypes were then selected from the respective lines. The same procedure was used in the second selection experi-

TABLE 44
SELECTION OF pod PHENOTYPES IN pod-K (EXPERIMENT II)

Generation	pod $\times$ pod				+ $\times$ +			
	Total	Percent- age pod unilat.	Percent- age pod bilat.	Total percentage pod	Total	Percent- age pod unilat.	Percent- age pod bilat.	Total percentage pod
F ₁	109	41.2	19.3	60.5	...			
F ₂	106	35.8	11.3	47.1	16	62.5	25.0	87.5
F ₃	84	35.7	16.7	52.4	133	35.4	6.8	42.2

TABLE 45
OFFSPRING OF DIFFERENT TYPES OF F₁ MATINGS FROM pod-M(3)124

	pod $\times$ pod			pod $\times$ +			+ $\times$ +		
	Total	Percent- age pod unilat.	Percent- age pod bilat.	Total	Percent- age pod unilat.	Percent- age pod bilat.	Total	Percent- age pod unilat.	Percent- age pod bilat.
Females.....	45	37.9	11.1	113	24.8	2.7	156	21.9	6.4
Males.....	27	48.0	7.4	121	14.0	3.3	196	14.3	0.5

TABLE 46
SELECTION OF PODOPTERA AND NORMAL PHENOTYPES IN pod-M(3)124 (EXPERIMENT I)

Generation	+ $\times$ +				pod $\times$ pod			
	Total	Percent- age pod unilat.	Percent- age pod bilat.	Total percentage pod	Total	Percent- age pod unilat.	Percent- age pod bilat.	Total percentage pod
F ₁	361	18.0	2.5	20.5	72	43.1	8.3	51.4
F ₂	74	31.1	5.4	36.5	67	55.0	10.5	65.5
F ₃	235	24.7	1.7	26.4	301	39.2	12.7	51.9
F ₄	508	19.7	5.1	24.8	158	38.4	15.2	53.6

ment. If the results of table 46 and table 47 are compared, the following points are in agreement: (1) after one generation of selection the penetrance values of the pod phenotype are always higher in the group of matings pod $\times$ pod. (2) Within the pod $\times$ pod groups selection does not increase penetrance. (3) In the + $\times$ + groups some selection against pod is shown to be possible. Although the total percentage of penetrance of pod was not greatly reduced by selection of normal phenotypes, some factors must still have been heterozygous and were eliminated in some of the + $\times$ + matings. This is shown by the example given on the next page.

In the first selection experiment, two sublines (1950 and 1958) were established in F_2 . In line 1958 incidence of pod was more than half of that in the line selected for pod. Line 1950, on the other hand, showed a marked decrease in penetrance in F_3 and subsequent generations. The percentage of pod was as follows:

	Line 1958	Line 1950
F_3	31.4	11.7
F_4	36.9	8.9
F_5	22.3	3.4

TABLE 47

SELECTION OF PODOPTERA AND NORMAL PHENOTYPES IN pod-M(3)124 (EXPERIMENT II)

Generation	+ × +				pod × pod			
	Total	Percent- age pod unilat.	Percent- age pod bilat.	Total percentage pod	Total	Percent- age pod unilat.	Percent- age pod bilat.	Total percentage pod
F_1	300	30.3	6.3	36.6	154	29.1	2.6	31.7
F_2	559	18.1	1.4	19.5	426	28.6	7.0	35.6
F_3	801	14.6	1.2	15.8	795	26.2	2.6	28.8
F_4	510	22.4	1.8	24.2	246	27.7	3.7	31.4

TABLE 48

VARIATION OF EXPRESSIVITY OF tet

Group	Mean pen- etrance	Individuals in tet classes						Mean expres- sivity
		I	II	III	IV	V	VI	
1. Five generations low-penetrance broods	36.6	234	192	89	31	4	2	1.9
2. All broods contained in fig. 25 . . .	45.4	1,243	709	411	10	11	6	1.7
3. Extracted high-penetrance broods (5 generations)	80.0	309	128	159	13	3	1	1.8
4. tet-100	100	84	44	121	39	38	6	2.8

2. CORRELATION BETWEEN PENETRANCE AND EXPRESSIVITY

A. TETRALTERA

In fig. 25 a large population of inbred tet broods which will be used also for the study of bilaterality of the tet effects is studied for correlation between penetrance and expressivity. The first impression is that there seems to be no correlation, except for the higher penetrance grades above 65, which show a slight correlation. It has been shown already that this population consists of different genetic groups which can be selected for different combinations of multiple tet factors. Among these the highest factorial combination (many tet factors including the powerful ones) can be extracted in homozygous condition, and it shows a constant penetrance between 60 and 90 per cent. Thus, the slightly ascending part of the curve is based upon a mixture of plus types of

genetically low penetrance and selected broods of high genetic penetrance above 60 per cent. If the latter were isolated, the correlation would look different. There exists, in addition, a 100 per cent penetrance line, tet-100, for which the mean penetrance and expressivity values are marked in the figure, showing a high correlation between them. We compare, therefore, in table 48, the actual figures for (a) the entire group used for the curve of correlation (no. 2), (b) for a set of extracted, homozygous high-penetrance broods (no. 3), and (c) for the tet-100 line (no. 4) and a special series of low-penetrance broods (no. 1). The three inbred lines of different penetrance show approximately the same expressivity. Actually, the lowest penetrance group

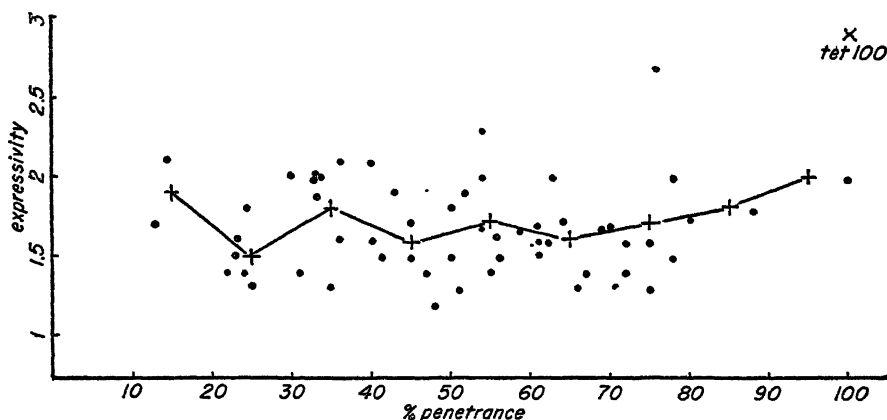


Fig. 25. Correlation between penetrance and expressivity in tetraltera.

(although not homogeneous genetically) has the highest expressivity. Thus, even if highly transgressive variation and the crudeness of the classification are taken into account, the genetic tet factors controlling penetrance below 60 per cent in the selected line and controlling different degrees of penetrance in different factorial combinations do not control a correlated expressivity. Only tet-100 shows a clear correlation between penetrance and expressivity; the tet factor specific for tet-100 also affects expressivity.

B. PODOPTERA G

The largest series of pod-G available for the study of the correlation of penetrance to expressivity is a group of inbred lines of isogenic pod-G presented above (table 30). This group is practically homozygous. Because of the low range of penetrance, only two groups are distinguished in table 49, namely, all broods (from 3 lines) with penetrance below 3 per cent and all above 5 per cent. The table shows that there is no difference in expressivity between the two sets. Comparing them with tet (table 48), we notice that, in both, the curve of variation is similar up to class V, which in tet is the haltere-like structure and in pod the real wingleg. But in pod, class VI is again represented by large numbers. This class is the one which in the records is called "parapod" (see description in Goldschmidt, 1945). Obviously, in pod-G this most extreme

TABLE 49

CORRELATION OF PENETRANCE AND EXPRESSIVITY IN ISOGENIC AND SELECTED pod-G OVER TWELVE GENERATIONS (LINES 14370, 14273, 14258)

Group	Individuals	pod class						Mean penetrance	Mean expressivity
		I	II	III	IV	V	VI		
1. All with penetrance below 3 per cent	12,763	117	101	65	11	8	70	2.9	2.7
2. All with penetrance above 5 per cent	4,445	96	75	42	7	2	48	6.7	2.6

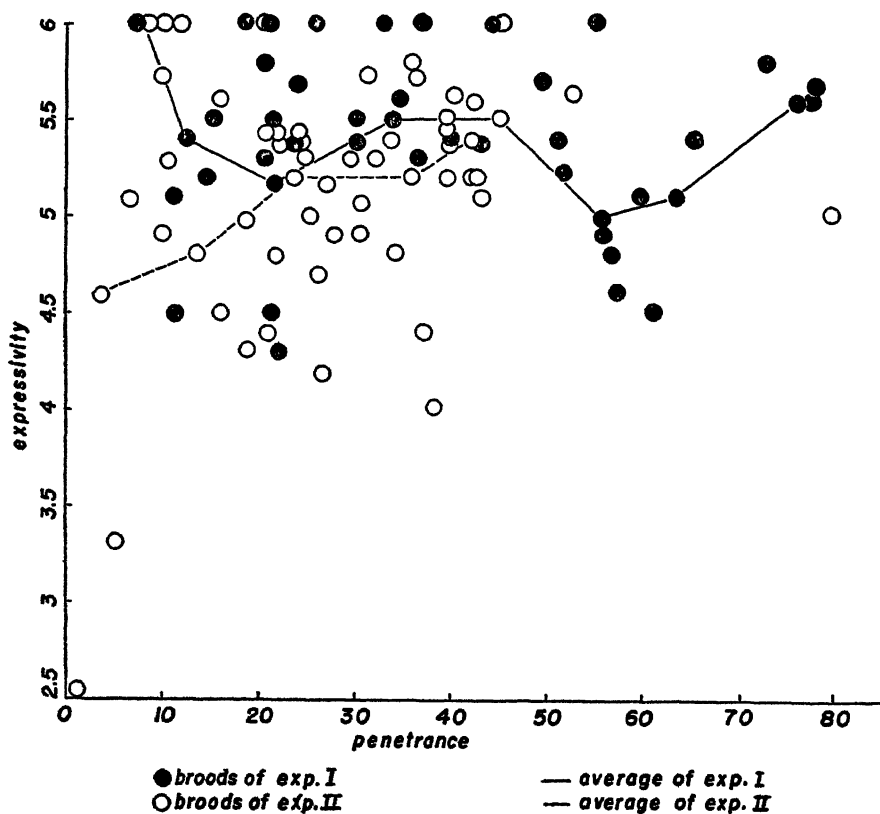


Fig. 26. Correlation between penetrance and expressivity in podoptera 124M(3).

change of the wing is more easily accomplished than some of the less extreme changes (IV and V). This is certainly not determined by specific genetic factors but must be connected with some critical period in the development of the disc at which a kind of (almost) all or (almost) none reaction takes place. In the stocks pod-K and pod-M(3)124, however, the great preponderance of class VI suggests that such specific factors may exist in the two stocks.

C. PODOPTERA M(3)124

All broods which were obtained in selection experiments I and II (tables 46, 47) were tabulated for the correlation of penetrance and expressivity; but because of the somewhat different genetical composition of the two series of selected matings, they are kept separate (fig. 26). Both series of broods show complete lack of correlation between penetrance and expressivity.

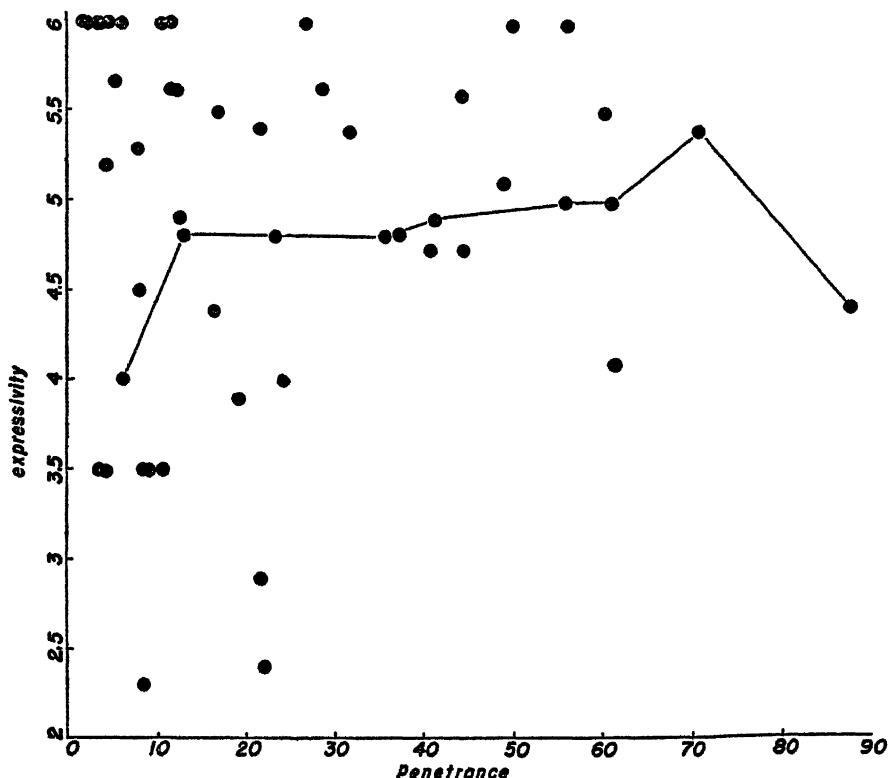


Fig. 27. Correlation between penetrance and expressivity in podoptera K.

D. PODOPTERA K

A graph (fig. 27) showing the relationship between penetrance and expressivity in the stock pod-K was compiled from a study of cultures of $+\times+$, $+\times$ pod and pod $\times$ pod matings taken from the stock. Again, there is no correlation between the two phenomena.

3. THE ASYMMETRY OF PODOPTERA

The phenotype of podoptera is always more or less asymmetrical. This means that most frequently only one wing (or leg) is affected, and that if both wings are affected they usually show different grades of expression on each side. Specimens with only one side affected, especially those of the higher classes

with much change in the tergites and bristles, might be interpreted as cases of somatic mosaicism. The following facts might lend themselves to such interpretation. If breeding is conducted only on a small scale, the offspring of asymmetrical pod flies may be normal, or again they may contain pod. One could conclude that this is due to somatic mutation, in the first case, and to somatic mutation affecting also the germ tract, in the second. Breeding from

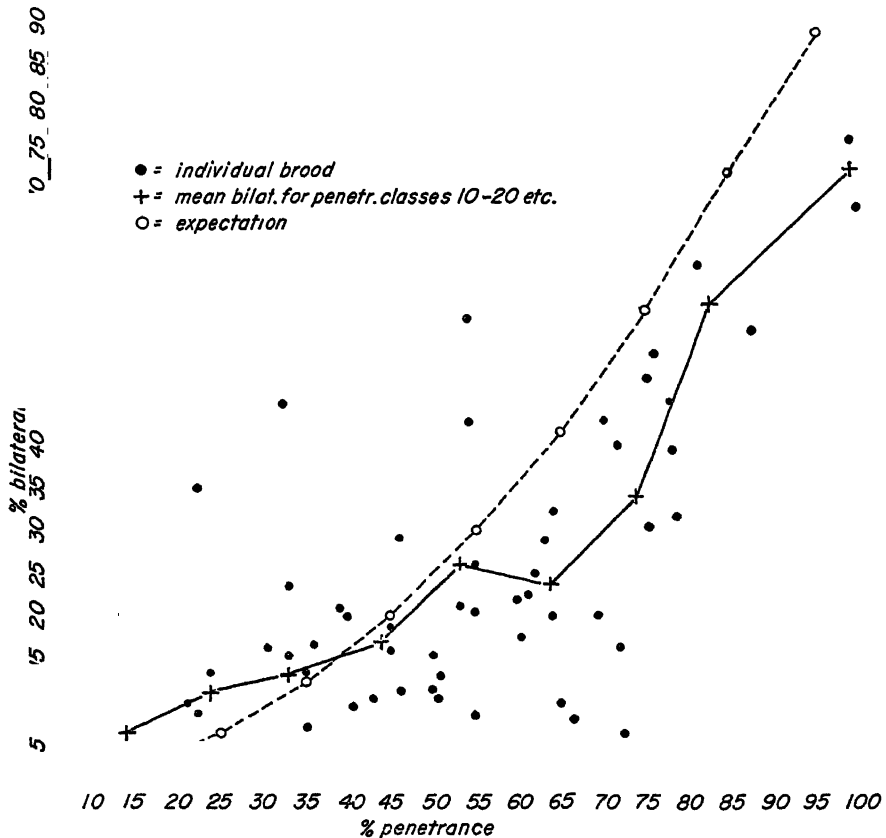


Fig. 28. Correlation between penetrance and bilaterality in tetraptera.

normal sisters again results in no podoptera flies, and it could be concluded that no mutation has occurred; if another brood gave podoptera, the conclusion would be drawn that somatic mutation had occurred. The alternative interpretation, which we consider to be preferable, is that low penetrance as well as asymmetrical expression of the character are purely developmental features within one and the same homozygous genotype. Because less well explored cases might be described in terms of somatic mosaicism (an error which probably has occurred frequently) an analysis of the situation is important.

Some descriptive data on asymmetry and its frequency in tet and pod-G are found in earlier publications by Goldschmidt (pod) and Vilee (tet). A

special investigation has been made for tet because the high penetrance in certain lines furnishes larger numbers. (Astauroff [1930] studied the asymmetry in the homoeotic mutant tetraptera biometrically and concluded that there is no right-left correlation. Gloor [1947] did the same for phenocopies of tetraptera.)

If the asymmetrical effect is based upon genetic mosaicism one should expect that all grades of expression (classes I-VI) contain the same frequency of bilateral effects. Further, the percentage of bilaterally affected flies among all pod specimens should be the same whether the brood shows a low or a high penetrance of the character. If, however, unilaterality and bilaterality are determined on a purely embryological basis without genetic mosaicism, we must assume that a low effect, shifting from normal toward abnormal development, is apt to act only on one side of the body, and that the probability of both sides being affected increases with the magnitude of the effect as well as with the percentage of affected flies (penetrance). (A general discussion of this situation from the point of view of physiological genetics is found in Goldschmidt, 1937, p. 222.) There is an additional possibility, namely, that bilaterality is an embryological phenomenon based upon the developmental features which result in more or less penetrance, but that special genetic modifiers influence the incidence of uni- or bilaterality. The proof or disproof would have to be derived from selection experiments. In the following presentation of data we deal only with the alternative unilateral vs. bilateral effect. The asymmetry of the bilateral effect will be discussed separately.

A. CORRELATION BETWEEN PENETRANCE AND BILATERALITY OF WING EFFECT

a. *Tetraltera*

Figure 28 shows the correlation between percentage penetrance of the tet effect and the percentage of bilaterally affected flies in 64 broods of a highly inbred tet line (by mass selection) in which penetrance varies from 10 to 100 per cent (the details of this line have been discussed in the chapter on selection). The total number of flies is: 5,579; number of tet: 2,533; number of bilateral tet: 597; mean number of flies in brood: 87. The individual average broods are represented by dots; the crosses signify mean percentage of bilaterals for the means of the penetrance groups 0-10, 10-20, etc. The exact numbers are found in table 50.

The curve shows a clear positive correlation, but considerable scattering of individual broods. The break in the curve in class 60-70 is caused by one extremely aberrant brood with the highest percentage of bilaterals of all, in the 50-60 class. The curve shows, further, an exponential tendency in that the percentage of bilaterals increases geometrically in the higher penetrance groups, in a manner comparable to a growth curve. Thus, these results favor an explanation of bilaterality as a phenomenon of embryological determination rather than one in terms of a special genetic basis. The form of the curve suggests at once the real reason for the correlation between penetrance

and bilaterality, namely, completely independent variation of the two sides of the body, i.e., lack of right-left correlation. If both wings vary independently in regard to penetrance, the probability that both wings are affected simultaneously is the product of the individual penetrances. In fig. 28, the theoretical expectations for values of bilaterality in case of right-left independence are calculated for the mean penetrances (in classes of 5 to 15 to 25, etc., per cent penetrance) and are entered as the broken curve. The fit is as good as can be expected and demonstrates that bilaterality is a function of independent variation of penetrance on the two sides of the body. This agrees with Astauroff's (1930) analysis of the similar situation in tetraptera.

TABLE 50
CORRELATION OF THE MEANS OF PERCENTAGE PENETRANCE WITH
THE MEANS OF PERCENTAGE BILATERALITY IN tet

Penetrance group	Number of broods	Mean penetrance	Mean bilaterality
10-20.....	4	14.8	6.3
20-30.....	7	24.0	10.9
30-40.....	12	33.7	14.0
40-50.....	7	44.0	16.7
50-60.....	11	53.5	27.9
60-70.....	11	64.2	23.3
70-80.....	8	74.8	32.0
80-90.....	2	87.5	57.3
90-100.....	2	99.3	72.7

b. Podoptera M(3)124

The material presented in graph 26 on correlation between penetrance and expressivity was also plotted for the correlation of penetrance and bilaterality. For both series the graphs show a positive correlation for penetrance and bilaterality (fig. 29).

c. Podoptera K

The positive correlation between penetrance and bilaterality found for tet and pod-M(3)124 was not clearly shown for pod-K by plotting the same data used for the determination of the relationship between penetrance and expressivity (fig. 30). However, there seems to be a slight correlation.

B. CORRELATION OF EXPRESSIVITY AND BILATERALITY

Expressivity of the wing effect can be measured in terms of the mean class value (classes I-VI; see above). If asymmetry of the effect is based upon a single embryogenetic process which pushes the action upon the disc sometimes beyond the threshold for both wings, and sometimes not, one would expect that bilaterality increases with the degree of expressivity.

a. Tetraltera

To test this hypothesis in tet the same series of broods as was used in the last section was tested for correlation between expressivity and bilaterality (except

broods in which the types had not been classified). Fig. 31 shows graphically the correlation between expressivity and bilaterality. Except for the right end of the curve, where the points are not significant because there was only one brood in each class, there is no correlation.

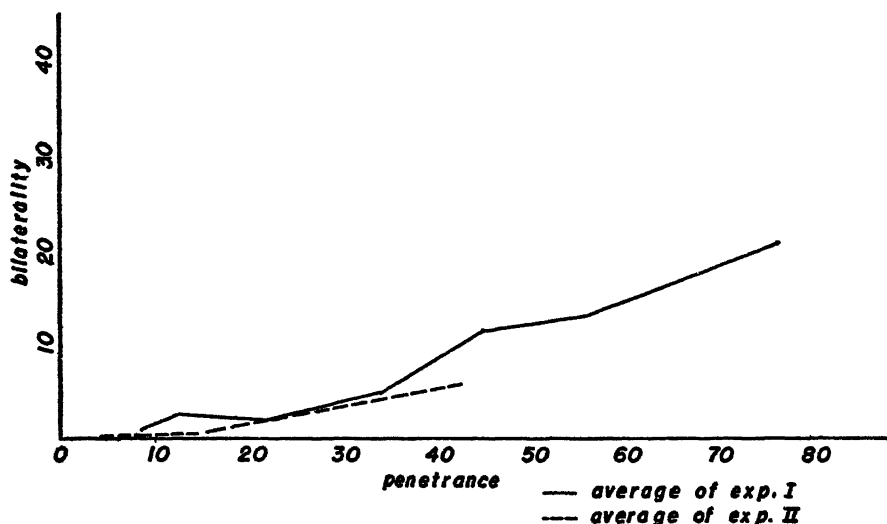


Fig. 29. Correlation between penetrance and bilaterality in podoptera M(3)124.

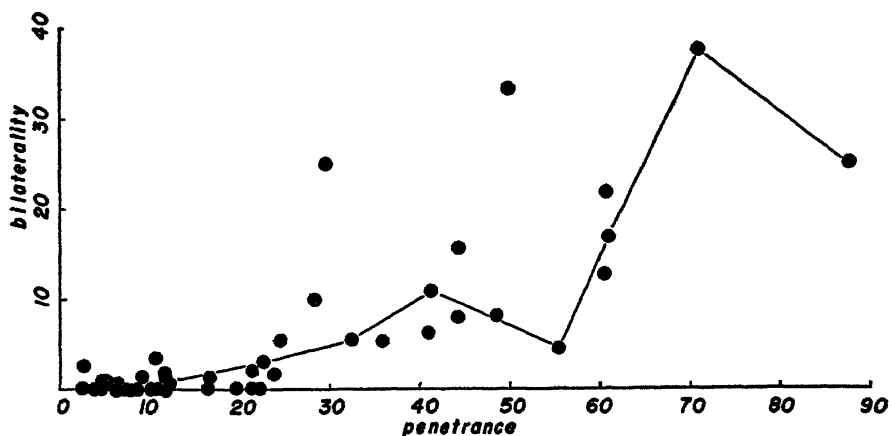


Fig. 30. Correlation between penetrance and bilaterality in podoptera K

But this negative result is deceptive. Actually, this inbred set showed very little variation in expressivity. It is typical for this line—the series under consideration contains 5 generations—that the tet flies show a rather low expressivity under standard conditions (see below: environment). The overwhelming majority of flies belong to the first 3 classes of expression, and the higher classes are represented only by few individuals. Among 2,390 unilateral

tet phenotypes 1,952 belonged to classes I and II, 411 to class III, only 10 to class IV, 11 to class V, and 6 to class VI. Classifying the bilaterals according to the highest class exhibited in one or both wings, we find in class I and II, 340; III, 138; IV, 3; V, 7, and VI, 1. In percentages of total tet flies we have:

	Class I/II	III	IV-VI
Asymmetricals	81.7	17.2	1.1
Bilaterals	69.5	28.2	2.3

This shows that a higher percentage of the higher classes occurs among the bilaterals. Thus, in a line homozygous for factors controlling medium pene-

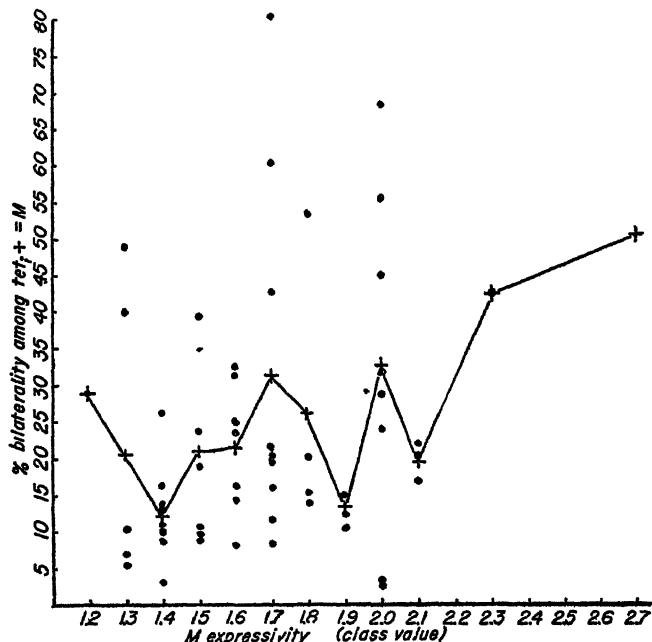


Fig. 31. Correlation between expressivity and bilaterality in tetralters.

trance of tet and a rather uniform low expressivity, the percentage of bilaterals is not correlated with the degree of expressivity within the lower classes of expressivity but is clearly correlated in the higher classes. This leads to the conclusion that, although the variability of penetrance in the line is not necessarily due to the variation of a developmental process responsible for the tet phenotype, the processes controlling expressivity and bilaterality are correlated or identical.

Another test for these relations is a comparison of mean expressivity in asymmetricals and bilaterals. The values 1.8 and 2.0, respectively, may again be interpreted to show a correlation between bilaterality and expressivity.

If our assumptions are correct, the reciprocal frequencies of asymmetry and bilaterality in the tet line are not controlled by genetic modifiers. The parent-offspring correlation may be used as a control. If modifiers for bilaterality are involved, a higher rate of bilaterals in the offspring of tet-phenotype

parents can be expected. In our experiment all the broods were bred either from phenotypically normal parents or from phenotypically tet parents. Therefore, we arranged the data for penetrance and bilaterality according to the phenotype of the parents:

	Normal Parents	Parents phenotype tet
Mean per cent penetrance	45.6	56.0
Mean per cent bilateral	20.7	26.0
Penetrance: bilaterality	2.2	2.2
Number of broods	24	34

It is clear that no genetic control of the frequency of incidence of bilaterality is involved within the limits of this test, and therefore bilaterality seems to have no genetic control of its own.

Evidence for genetic control of bilaterality was, however, obtained for the line with a constant 100 per cent penetrance of tet (see the special chapter on tet-100). One such test made simultaneously with the others of the group just reported showed the following results:

Totals	Asymmetricals	Bilaterals	Penetrance	Bilaterals	M expressivity (class)
116	27	89	100	76.7	3.5

Penetrance and bilaterality show a very high positive correlation, and so do bilaterality and expressivity, in contrast to the lower penetrance tet lines. This correlation also becomes evident in the individual bilaterals, of which 11 in 89, or 12.3 per cent, have reached the highest grade of bilaterality, namely, both wings class V (halteres). The data from this line with 100 per cent penetrance prove the correctness of the assumption; e.g., the asymmetry phenomenon in tet is not based on any kind of mosaicism but on developmental features involving quantities of active substances and thresholds, as will be discussed below.

An attempt was made, of course, to select for bilaterality; this is difficult because pair matings are usually sterile (because of premature death of one partner). In view of the difficulties with pair matings of the extreme types, another experiment with mass selection was performed. The material for this experiment was taken from another line of tet. We mentioned the establishment of the tet-100 line, which was produced (see below) by inserting an additional tet factor into the first chromosome of a high-grade tet line. This tet-100 line was kept successfully as a stock. But when, many months later, flies from this stock were taken for a mass selection experiment, it was found that the stock had changed, supposedly by mutation, so that again a variable number of phenotypically normal flies appeared. The completely consistent results over 5 generations of selection carried out in different ways are presented in the pedigrees below:

PEDIGREES

1. Selection for bilaterals

F ₁ tet 91.2 per cent, bilat. 48.6 per cent	
F ₂ tet 86.4 per cent, bilat. 50.2 per cent	
F ₃ a. tet 94.0 per cent, bilat. 67.4 per cent	F ₃ unilat. tet 81 per cent, bilat. 33.3 per cent
b. tet 37.4 per cent, bilat. 5.3 per cent	cent
F ₄ from a. tet 84.7 per cent, bilat. 48.2 per cent	

2. Selection for unilaterals (and bilaterals)

F ₁ unilat. tet 68.3 per cent, bilat. 17.1 per cent	
F ₂ unilat. tet 55.1 per cent, bilat. 14.3 per cent	F ₂ from bilat. ² tet 84.7 per cent, bilat. 53.2 per cent
F ₃ unilat. tet 54.5 per cent, bilat. 19.2 per cent	F ₃ unilat. × bilat. tet 74.6 per cent, bilat. 45.5 per cent
F ₃ from bilat. tet 54.9 per cent, bilat. 15 per cent	
F ₃ unilat. × bilat. tet 48.5 per cent, bilat. 3 per cent	
F ₄ unilat. tet 48.8 per cent, bilat. 10.5 per cent	F ₁ bilat. ² tet 80.5 per cent, bilat. 46.3 per cent
F ₁ + × + tet 32.8 per cent, bilat. 8.4 per cent	bilat. ² tet 81.8 per cent, bilat. 62.8 per cent
F ₄ unilat. tet 44.4 per cent, bilat. 8.4 per cent	
F ₃ unilat. tet 90.4 per cent, bilat. 38.7 per cent (Bd-mut.)	F ₂ bilat. ² tet 90 per cent, bilat. 60.3 per cent

This experiment shows clearly the positive correlation of penetrance and bilaterality (fig. 32). Straight selection for bilaterality yields broods with more than 80 per cent penetrance and $\pm$ 50 per cent bilaterals. But one low-penetrance and bilaterality brood appeared in F₃ of this mass selection, showing that there were still genetically low penetrance combinations with transgressive variability present in the selected group and selected out by chance in this one case. One F₃ from unilaterals gave a high penetrance although the bilaterals fell below the average. In the straight selection for unilaterals F₁–F₄ a constant penetrance of $\pm$ 50 per cent tet and bilaterality of $\pm$ 14 per cent was maintained, also in the F₄ brood bred from bilaterals. In F₁ a side line was started from bilaterals (right side of pedigree); this line maintained through 4 generations the high penetrance and bilaterality of a selected bilateral line. Selections of bilaterals made in F₂ showed the bilaterals to be plus individuals of the genetic lower penetrance group in all three combinations made (bilat. × bilat., bilat. × unilat., + × +). A strange feature appeared in F₅ of the unilateral selection line. Here a dominant mutant, a Bd-allele, appeared, and simultaneously penetrance and bilaterality increased to the high level. The case will be discussed below with the genetic analysis of Bd in relation to pod. Thus, it may be safely concluded that bilaterality in tet is not based upon special genetic modifiers and is not correlated to penetrance in the lower penetrance lines. But, as the tet-100 line shows a correlation between penetrance and bilaterality, the specific genetic features of this line control in some way the developmental processes responsible for bilaterality.

We have not mentioned the relation of expressivity to sex; this will be taken up in the discussion of the relation of sex to penetrance, in the section on the Y chromosome.

b. Podoptera H

In pod-H, between 10 and 15 per cent of all podoptera flies were bilaterals (table 9). Among 61 bilaterals, 26 had wings affected in the same way, i.e., they belonged to the same class, although even in these cases, with the exception of class I, the phenotypes were not identical (class II, for example, is made up of 5 subclasses, with rather different phenotypes). The majority of bilaterals (35) had one wing of one class and one wing of another class; and the more extreme types were never of the same class. These data show that there is no right-left correlation in the expression of the pod effect in pod-H.

The penetrance in pod-H is so low that a detailed study of the correlation between penetrance and expressivity and bilaterality was not undertaken. There is no reason to assume that the results would differ from those reported for tet or the other podoptera stocks.

4. MODIFICATION OF PENETRANCE

Numerous cases are known of environmental factors which increase the amount of penetrance. An extreme case is that of the absence of phenotypic

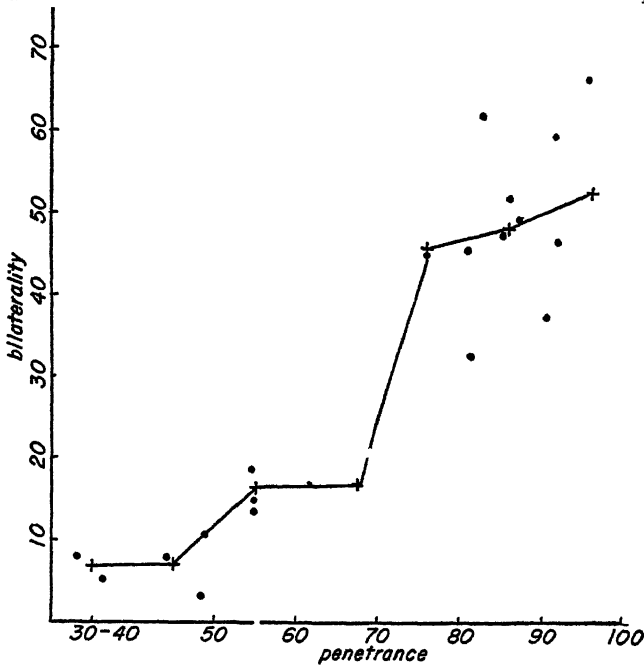


Fig. 32. Correlation between penetrance and bilaterality in tet-100.

effect of a mutant except under special environmental conditions. As a full understanding of the podoptera effect requires also the knowledge of the response to environmental changes, experiments were made to test high and low temperatures, crowding, and prolongation of developmental time. Some experiments were also designed to test the critical time in development.

A. TETRALTERA

Temperature experiments on tet were carried out in our laboratory by Villee and were reported (1942, pp. 139-146). But, as Villee dealt with unselected stocks, his data are only of comparative value, although they make it clear that low temperature increases penetrance. No definite sensitive period could be found. The greatest effect was produced by continuous cold. The effect of heat shocks also could not be related to a definite period of development. The details of the cold experiments, however, indicated that a sensitive period may be found during early embryonic development, as expected from morphology.

(Gloor, 1947, found the sensitive period for phenocopies of tetraptera to be in the egg stage.) Experiments did not yield positive results. Increasing the time of larval development by Nipagin or starvation had very little effect.

These experiments did not encourage much further work. But certain observations showed that there are factors which increase penetrance considerably. In mass breeding of highly selected tet lines with an average penetrance of about 50 per cent (in single-pair bottles) only very low penetrance is found. It is most probable that this is not a phenomenon of penetrance but selection due to crowding, since tet flies are always less viable than normal ones, as stated above. Table 51 gives an example of this type of behavior. If

TABLE 51
PENETRANCE IN MASS AND PAIR BREEDING OF tet

Number of P	Percentage penetrance of parents	Penetrance of F ₁ mass	Number of F ₁ mass	Penetrance of F ₂ pairs	Number of F ₂	F ₂ is average of n pairs
99.....	35	1.3	223	52	447	4
145.....	39	2.2	409	61	288	4
72.....	24	3.3	124	24	258	2
51.....	47	1.0	303	50	126	1
123.....	14	2.4	256	..	...	..
84.....	27	0.3	401	62	181	2
141.....	31	...	600	68	461	4
75.....	61	0.9	111	45	126	1
133.....	56	0.8	253	58	358	2

stock bottles become old and dry and the flies grow smaller and smaller, the number of tet flies, and especially the expressivity, increases so that a majority of individuals are class V and bilateral. This observation applies also to low-penetrance stocks in which practically no tet flies are seen ordinarily, although they will appear in old bottles. It seems that here are realized undefined conditions of starvation which could not be isolated in systematic starvation experiments performed by Villee. It would be worth while to perform experiments with specific vitamin deficiencies.

B. PODOPTERA G

As reported above, pod-G has always a very low penetrance which is higher in isogenic stocks than in selected, presumably homozygous, ones. The temperature experiments with pod-G, including also high temperature shocks (over 35° C.) and low temperature shocks (12° C.), are tabulated in table 52. The results, as tabulated, show such irregularities that, within the limits of the experiment, no positive information has been gained.

We described, above, the negative influence of crowding upon penetrance in tet. We made some more detailed experiments for pod-G, which originally were meant to produce better penetrance for genetic analysis. The procedure was to compare (of course under otherwise identical conditions) ordinary one-pair bottles in which the parents remained until the first offspring hatched

with bottles in which eggs had been laid for 24, 48, and 72 hours, comparing the penetrance of podoptera with the total number of individuals per brood. All experiments were performed with the isogenic line. Table 53 contains the summarized results for all bottles. This table shows clearly a higher penetrance in bottles with about half the average number of flies than that of the controls,

TABLE 52
TEMPERATURE AND PENETRANCE IN pod-G

Experiment	Line	Flies		Percentage penetrance	Remarks
		+	pod		
1. Control 25° a.....	pod-G inbred	689	3	0.42	Selected for pod phenotype
2. Control 25° b.....	pod-G inbred	5,418	40	0.74	
3. Control 25°.....	pod iso.	5,475	176	3.2	
4. 16° C a.....	pod-G inbred	3,151	27	0.85	
5. 16° C. b.....	pod-G inbred diff. line	3,624	24	0.66	
6. 16° C. c.....	pod-G inbred diff. line	902	3	0.33	
7. 16° C.....	pod iso.	1,232	34	2.7	
8. Room = ca. 20°.....	pod iso.	3,062	40	1.3	
9. Room = ca. 20° a.....	pod-G inbred	252	2	0.79	
10. Room = ca. 20° b.....	pod-G inbred same line as 6	370	1	0.24	
11. 25°, 38.5° 3h after 48h.....	pod-G inbred	312	..		
12. 25°, 38.5° 3h after 48h.	pod-G inbred	576	5	0.86	
13. 48h 25° then 12°.....	pod-G inbred	135	..		
14. 96h 25° then 12°.....	pod-G inbred	145	..		
15. 24h 25°, 24h 35°, then 25°..	pod-G inbred	1,068	4	0.37	
16. 48h 25°, then 35° during 48-72h.....	pod-G inbred	749	1	0.13	
17. 72h 25°, then 35° during 72-96h.....	pod-G inbred	413	1	0.24	
18. 35° during 0-96h.....	pod-G inbred	988	4	0.41	

although there is no significant difference between the 24-, 48-, and 72-hour broods. The first two columns of the controls show the same results as reported above, namely, that in the isogenic line, breeding from pod phenotypes produced a higher penetrance than breeding from normal phenotypes.

In order to test whether this over-all correlation holds also for the individual broods, the correlation between numbers per brood and percentage of penetrance was checked for all broods of this otherwise homogeneous series. There was no correlation in the large broods. These results may indicate that there is an inverse correlation between penetrance and size of broods, but that with increase of the size of the brood beyond 70-100 individuals another factor comes into play which again favors penetrance more than crowding suppresses it. This factor might be something relating to a balanced growth of yeast, or

it could be a similar nutritive factor, which acts as a selective agent in favor of the pod phenotype.

The increase of tet flies in old bottles suggested that the manifestation of the tet phenotype might be associated with longer developmental time. To test this for pod-G, counts were made daily from the tenth to the eighteenth day in the isogenic line. If the difference between emergence of normal and pod phenotypes among brothers and sisters was considerable, all pod flies should

TABLE 53
COMPARISON OF PENETRANCE OF ISOGENIC pod-G IN DIFFERENT DEGREES
OF CROWDING

No.	Egg-laying days	Breeding	Average number per bottle	Totals		Percentage of penetrance
				+	pod	
1	control 10.....	+ × +	188	555	9	1.6
2	control 10.	pod × pod	134	521	15	2.8
3	control 10....	All	157	1,076	24	2.2
4	1	All	48	839	31	3.6
5	2....	All	167	319	14	4.2
6	3.	All	91	873	37	4.1
7	1-3.....	All	70	2,031	82	3.9

be crowded into the later days of hatching. The actual result did not indicate any effect of sufficient size to warrant a study with more refined methods. The actual counts were (penetrance about 2 per cent):

Day of hatching at 25° C: 10, 11, 12, 13, 14, 15, 16, 17, 18

Number of pod phenotypes: 4, 8, 3, 5, 4, 4, 5

C. PODOPTERA K AND M(3)124

The reduction in developmental time and the increase in the rate of development, processes which results from exposure to high temperature (Child-1935) caused a very pronounced decrease in the manifestation of podoptera in pod-M(3)124 (table 55). This finding suggested that one factor in the production of podoptera in Minute stocks is the prolongation of larval development which is characteristic for the Minutes (Brehme, 1939, 1941, 1941a, Dunn and Coyne, 1937). From the data to be reported later (p. 227) concerning the production of the podoptera phenotype by the isolated mutant Minute(3) 124 and the occurrence of pod in substitution lines lacking the Minute chromosomes, it is evident that developmental delay alone is not the decisive factor for the production of the podoptera phenotype in high-penetrance podoptera lines. Since the Minute chromosome is, however, probably the most important podoptera-producing chromosome of the stock pod-M(3)124, the developmental delay produced by this mutant may provide the necessary physiological conditions under which other podoptera factors might operate.

Attempts were therefore made to delay development, by environmental agents in a number of stocks, and to determine whether podoptera phenotypes

could be made to appear in higher numbers in low-penetrance podoptera stocks, and whether still further increases in the penetrance of podoptera could be induced in the high-penetrance stocks.

The agents commonly used to delay development in genetical experiments are low temperature, partial starvation of larvae, and combination with other mutants known to prolong the developmental period. Dunn and Mossige (1937) differentiate between the delaying agents that prolong the period of development without distorting the growth rate and others which induce some qualitative change in some developmental reactions in addition to increasing the period of development. The first group includes low temperature and the giant mutant (Bridges and Gabritschevsky, 1928). The larvae grow for a longer time at essentially the same rate, and the results are larger larvae and larger adults. Minutes and larval starvation belong to the second group. Although the period of larval development is prolonged, the adult flies are smaller because certain essential growth factors are not supplied by the medium, or because some change is induced in the developmental processes during larval life. Dunn and Coyne suggest that a change in the growth rate of the Minutes would account for the small size of Minute flies even though they have grown for a longer period than the wild type, but Brehme (1941a), on the basis of a detailed study of time relations during development of Minute larvae, states that her experimental results do not support this assumption. Instead, she suggests a qualitative difference of growth processes.

The addition to the medium of chemicals which produce partial starvation of the larvae might therefore be expected to produce an effect which resembles to a certain extent that of the Minutes. Child and Albertowicz (1937) and Child (1939) reported that the addition of Nipagin (methylparahydroxybenzoate) to the medium prolongs development by several days. They attribute the action of Nipagin to its inhibition of the growth of yeast cells on the medium, thus producing partial starvation of the larvae.

Nipagin, in a concentration of 0.2 per cent by weight, was added to the standard laboratory food (formula by Glass, D.I.S. 16). The autoclaved yeast was added to the food, as usual, and all cultures were also seeded with a drop of live yeast suspension. A number of different stocks were raised on this medium. No accurate timing of the delay produced by the action of Nipagin was made, but it was observed that in all cultures containing the chemical, pupation started about two days later than in the control bottles. The data which were obtained in this experiment are summarized in table 54. The first group recorded in the table are F_2 flies of the low-penetrance podoptera line which was described in the selection experiment of pod M(3)124. Penetrance of podoptera was three times that obtained in the controls. The next group is the line of pod M(3)124 which was selected for podoptera factors. Here penetrance of podoptera was again increased in the experimental series as compared to the controls. Partial starvation also produced an increase in the penetrance of podoptera in pod-K (no. 4). No such increase could be detected for pod-G or for a laboratory stock of M(3)w which shows very low penetrance of podoptera. Incidence of pod in Canton Iso '44 is so low that the observed in-

explained by stating that in the first case only the developmental time is changed, whereas in the second case the larvae grow less efficiently because of lack of essential nutrients.

The influence of high temperature on incidence of podoptera phenotypes in pod-K could not be tested. Forty broods were raised at 28° C., but only 24 flies hatched in the whole series. All were normal, but the number is so small that no conclusions can be drawn.

It seems that Rapoport (1942) also performed experiments with starvation and prolongation of developmental time and obtained similar results, although he gives no details.

TABLE 56
INCIDENCE OF PODOPTERA PHENOTYPES IN pod-K AT 16° C.

Phenotypes mated	Total	Percentage of pod
pod × pod.....	882	5.9
pod × ro-l no-l.....	113	5.3
ro-l no-l × pod.....	262	1.8
ro-l no-l × ro-l no-l.....	797	2.2
Σ.....	2,054	3.9

5. SUMMARY OF PART II

1. A selection experiment for penetrance of the tetraltera phenotype is described and analyzed. It shows that high or low penetrance is genetically controlled. In the case of lower penetrance, normal individuals may be genetically identical with those of tet phenotype. Higher penetrance is based upon the presence of a number of tet factors which can be selected out in homozygous condition up to a penetrance of about 80 per cent. The intermediate penetrance values are the result of different combinations of tet factors. From the selection experiments alone, it cannot be decided whether only recessive factors are involved, and whether different factors have a different potency of action. Actually, very low and very high penetrance lines can be extracted by selection. The total results favor the assumption of a rather small number of multiple factors of additive or multiplicative effect controlling penetrance of tet.

2. Selection for penetrance of podoptera in different pod-G lines raised the percentage from 0.4 to about 1.5 per cent, indicating a similar situation as in tet but without any powerful pod factors. Penetrance was further increased by making the stock isogenic, which amounts to a quick selection experiment. Even in the isogenic line a significant increase of penetrance by selection was possible, which might have been due either to the not-isogenic fourth chromosome and part of the third or to relatively frequent mutation of pod factors.

3. An extensive selection experiment was carried out with pod-H in which a significant increase of penetrance from 1.2 to 5.7 per cent was secured. The detailed analysis points to selection of multiple pod factors. Here, as in tet,

accumulation of pod factors results in loss of viability, complete sterility being reached after some generations. In this line also, selection experiments with parents of different phenotype (normal, podoptera, gynandromorph) were made which furnished the proof that in pod-II gynandromorphism is a pleiotropic effect of one pod factor. Selection was also exercised in isogenic lines which first had low penetrance, which rapidly was increased by selection; but after a few generations complete sterility ensued. In this case it seems more probable that in the isogenic selected line new pod factors (or more potent ones) appeared by mutation. Since sterility increased with increase in penetrance of podoptera, it is possible that the mutant factor or factors were simultaneously sterility factors.

4. In pod-K the podoptera phenotypes have a reduced fertility which makes selection experiments difficult. But it was possible to increase penetrance to more than 50 per cent, at which level homozygosity of the pod factors seems to have been attained. In pod M(3)124 also a certain amount of plus and minus selection was possible, indicating a similar genetic basis as in the other lines, i.e., multiple factors controlling penetrance.

5. Expressivity as measured by the classes of the phenotypic effect is not correlated to penetrance in tet; but the factor controlling the 100 per cent penetrance in tet-100 also increases expressivity. There is no correlation between expressivity and penetrance in the different pod lines. But the highest class of expression (type VI) is generally more frequent whatever the penetrance in pod-G and pod-K. This must be based upon specific embryonic conditions.

6. The pod phenotype is most frequently asymmetrical, but a certain percentage of flies show both sides affected. There is a strict correlation between penetrance and bilaterality, showing that the same developmental feature which controls penetrance of the character also controls the bilaterality of the effect in the same progression. The form of the curve of correlation in tetraltera suggests that the correlation is the result of complete right-left independence of variation. Bilaterality and expressivity are also correlated in tet, both being obviously determined by the same embryological feature. Parent-offspring correlation agrees with this conclusion. Selection for bilaterality agrees also, so far as it is successful, when high penetrance is selected simultaneously. As far as the data go, the results with pod lines agree with those obtained on a larger scale for tet.

7. Penetrance is to a certain extent dependent upon environment, but less so than might be expected. Crowding decreases penetrance in tet, possibly because of selection against the weaker tet phenotype. In old dry bottles penetrance is increased, possibly by physiological selection in favor of tet (some vitamin deficiency is suspected to be the selecting factor). In pod-G it was found that in large broods penetrance is not affected by the size of the brood; in medium-sized broods penetrance increases moderately in inverse ratio to brood size, and in small broods this correlation is pronounced. Obviously, two environmental factors acting in opposite directions are involved—crowding and a nutritive feature.

8. In pod-K, penetrance is decreased by low temperatures and increased by agents increasing the length of time of development, like starvation. In pod-M(3)124, however, low temperature is rather ineffective, whereas starvation experiments increase penetrance considerably.

6. PHENOGENETIC DISCUSSION

The facts presented thus far require an interpretation in respect to the phenogenetic aspects of the podoptera effect. The following points should enter such a discussion. There is first the general problem of homoeosis. If the mutant aristopedia produces a tarsus instead of an arista (and all transitions between the two), and if the mutant proboscipedia makes a proboscis assume the shape of an appendage ending in two tarsi, the problem of determination, in a general way, is the same as in the case of a wing being changed into a leg-like structure. Thus, the fundamental problems of embryonic determination should be similar in all homoeotic mutants. In the case of tetraltera and also that of tetraptera an additional problem presents itself, namely, the transformation of wing into haltere and vice versa. In podoptera (and also in tetraltera to some extent) still another relationship is encountered provided that the antenna can be classified as a ventral appendage. The homoeosis involves dorsal and ventral appendages, which usually are not considered to be homologous (see, however, discussion in Goldschmidt, 1945). In addition to these problems, more detailed questions arise: (1) What is the relation between tetraltera and podoptera? (2) Why can the leg-type appendage be formed from a whole wing disc as well as from the anterior part alone? (3) What do the different degrees of penetrance and expressivity, symmetry and asymmetry, and their respective correlations mean in terms of embryonic development?

Complete answers to these and similar questions cannot be given at this stage of our study, because experiments with the larval discs have not been performed as yet. But the data, as they stand, permit at least a preliminary analysis if the facts are compared with those found in homoeotic mutants on which experiments have been performed using the techniques of the experimental embryologist. Thus far the best-explored homoeotic mutant is aristopedia, in which the arista of the antenna is transformed more or less completely into a tarsus. Balkaschina (1929) had reported that normally the antenna differentiates late in larval life and the leg discs differentiate early, but that in aristopedia the antenna also differentiates early. From this, Goldschmidt (1937) concluded that in the early stages of leg development an evocator exists which acts upon any disc which is in the reactive condition. Braun (1939, 1940) and Vilee (1944) performed many experiments which gave results that seemed to agree with this hypothesis, while Waddington (1940) proposed less-simple mechanisms. The recent work of M. Vogt (1946, 1947) however, disproves Goldschmidt's explanation in its simple form. She showed that Balkaschina's data are not correct. Actually, the first visible differentiation (segmentation, i.e., visibility of the later jointed segments of the organ) occurs first in the antennal disc and only much later in the leg discs, both in normal and *ss*^{*} (aristopedia) flies. The penetrance and expressivity of the

tarsus character in the antenna is increased with lower temperature, and the effect upon the arista increases from proximal to distal. There is a temperature effective period of such an extent that even at the time of pupation an effect can still be produced, i.e., the determinative alternative is still labile, although the effect decreases with the progress of growth. Thus the tarsal segments of the antenna as well as the true leg segments differentiate late in aristopedia, but one early difference is visible; the tip segment of the antenna which transforms into a tarsus is larger from the beginning. In the leg disc the tarsal segment (which looks very much like the arista segment in the antennal disc) segments first, and the proximal leg segments differentiate later. Thus, in both organs there is a kind of gradient from distal to proximal; but this cannot be simply a determination stream resulting in irreversible determination, as the success of temperature experiments in aristopedia throughout the larval time shows.

Miss Vogt discusses some possible explanations: The antennal disc may always contain antennal and leg potencies. In the antenna of the wild-type fly only the antennal inductor is induced in quantities greater than the threshold requirement, whereas the *ss*^a mutant increases the tarsus inductor to threshold quantity; or the mutant *ss*^a produces a primary extra growth of the distal segment of the disc (the "end knob"), as observed, which produces a higher or earlier reactivity to the tarsus inductor. This would then constitute a probable modification of that proposed earlier by the senior author.

In subsequent papers Miss Vogt (1946, 1947) found that the transformation of the arista segment into the tarsus is linked with intensive growth of this segment. To test whether this relation is a causal one she treated explanted discs with colchicine in order to stop growth for some time. After reimplantation, an arista was formed instead of a tarsus. Waddington (1942) and Villee (1945, 1946) already had assumed that there must be a relation of growth to the type of differentiation. The fact that a completely unspecific interference with growth produces a specific differentiation suggests that the action of the mutant aristopedia is also an unspecific effect upon growth of the arista segment. This again, in our opinion, would mean either that a leg inductor, available in the disc, acts, if a definite growth pattern prevails, or that the growth pattern makes the segment susceptible to the action of a generally available leg inductor of the type that was formerly assumed by the senior author. Unspecific growth alone could hardly produce the specific tarsus-producing effect.

Miss Vogt does not discuss her interpretation in regard to penetrance and expressivity. Exposure to cold in the temperature-effective period increases the higher, tarsus-like classes of expression, which means that at this time the tissue of the end knob still has the potency to react at different rates and (or) to a different extent to the tarsus inductor; or to produce varying amounts of it. The rate of growth, slowed by cold, has something to do with this effect (stoppage of growth by colchicine had the opposite effect). In addition, the later the action of cold, the lower the expressivity; which means that the lability of determination is narrowed down to lesser and lesser effects with time within the labile period. Since, in case of low expressivity, the basal part of

the antenna shows a tarsus-like structure for the longest time while the distal part is arista-like, one might think that in aristopedia there is a determination stream for arista starting distally (and one for tarsus in the opposite direction). But the distal end can still become tarsus at the end of the labile period, as reported above. Miss Vogt therefore cannot accept such a determination stream. However, the order of expression—basis only tarsus-like, basis and tip tarsus-like (classes 1–5)—requires some gradient of determination, although it is not irreversible determination. The fact that high classes cannot be produced any more after a certain time links this gradient either with a gradient of growth from basis to tip, or with a superimposed gradient of irreversible determination in the same direction.

The question now arises how the homoeotic wing changes may be compared to and analyzed by comparison with the homoeotic antennal changes. In spite of the work of Chen (1929), Auerbach (1936), and Waddington (1940a) our knowledge of the early development of the wing is still insufficient. But it might be stated that in an early stage the wing disc might be roughly compared to the antennal or leg disc, since in all a crude subdivision into two or three segments becomes visible. The distal wing-disc segment later becomes the membranaceous wing. If we imagine that such a wing disc of the prepupal stage continues to differentiate without the end segment growing into the wing membrane, the result would be very similar to an early antennal or leg stage, namely a trisegmented palpus with a swollen distal segment. This palpus would be morphologically (i.e., in the gross structure) similar to a haltere, the haltere stage of tetraltera and—in a somewhat earlier or reduced condition—with class VI of the podoptera series which we found to be outside of the typical trend of variation of the other classes. Thus, in a general way, the basic structure of an antennal, wing or leg disc is the same for all of them. Therefore the transformation of any one into another is a less startling feat than might appear from the very different end products. (This analysis does not touch a further problem, namely, the differentiation of a tarsus in proboscipedia and aristopedia and absence of tarsus in podoptera.)

In the case of aristopedia, the analysis of the problem of determination required assumption of a leg inductor. Whether this was produced locally and always present in each disc, as assumed by Miss Vogt, or whether it did not originate locally and acted upon whatever tissue was ready for it, cannot be stated with certainty. If Miss Vogt's assumption is correct—and no other explanation appears likely—the wing disc may also contain (or be furnished with) a leg inductor. Therefore, the general situation in regard to the mechanism of determination of pod must be similar to that in aristopedia, in that normally the leg inductor in the wing disc is present in subthreshold quantities and that the pod factors increase its amount or its activity to a quantity above the threshold, or, vice versa, interfere with the action of the wing inductor. If this is true—and we cannot see that the conclusion can be avoided—the anlagen of all the segmental appendages have not only the same basic morphological structure, namely a trisegmented palpus, but contain also the leg inductor, whatever this is.

Again, the feature that was discussed for aristopedia becomes visible—the decisive influence of the pattern of growth. Growth of the tip segment of the wing anlage is needed to produce a membranaceous wing. Lack of growth is the first requisite for forming a haltere or a leg of the tetraltera type or of class VI of podoptera (but not of a tarsus, as in aristopedia). Thus, we see a relation of homoeotic development to the growth pattern comparable (not identical) to that found in aristopedia, and we may therefore assume that here, as there, the primary action of the tet factors is an unspecific effect upon growth with the consequences discussed above. It is in agreement with this conclusion that the cumulative action of the multiple tet factors is of an exponential order (as will be shown below), which is typical for growth curves. It may be added that the behavior of the mutant tetraptera, in which the haltere forms a wing with all intermediate stages resembling exactly those formed in the opposite order in tetraltera (Astauroff, 1929), fits well into the whole picture.

These general considerations on determination must now be extended to the specific features of tetraltera and podoptera. These features are: (1) The difference between tet and pod development, namely the transformation of the entire wing anlage into a haltere or leglike structure in tet, the transformation into a leg of only the costal part of the wing in pod. (2) The quadripartition of the pod wing. (3) The possibility (in pod) of more or less normal wing development of the three posterior wing parts, while the first one is transformed into a leg. (4) The fact that occasionally the fourth section (the alula) may form a palpus-like structure. (5) The observation that there is a certain tendency in tet to duplicate the posterior part of the wing, and in pod there is a tendency for duplicating the anterior, costal part. (6) All the data on penetrance, expressivity, and asymmetry. (7) The rare cases in which a pod wingleg may look like a tet wingleg of class V; and, more frequently, that a tet wingleg, of class VI resembles a pod wingleg of class V, although with differences in detail.

Let us first consider the difference between typical tet and pod on the basis of the facts of development discussed above. The situation for tetraltera is rather simple if the assumption is correct that the wing disc during the period of labile determination is morphologically a trisegmented structure comparable to that of the antennal disc. If the tet factors slow down the growth of the end segment relative to the speed of differentiation, this segment remains as a pear-shaped bladder which has (aside from the specific sense organs) the typical morphology of a haltere. The classes I–V are clearly caused by a different time of onset of the action of the tet factors, i.e., when wing growth and differentiation have already advanced to successive stages during the period of determination. If determination of the wing blade is practically ended, the stoppage of growth can affect only the later processes of determination, like venation and formation of the wing joint. Expressivity is thus a function of the time at which tet begins to act in the labile period, i.e., earlier action, higher expressivity. Penetrance (genetic and environmental) therefore means a variation in respect to the time of action of the tet factors (stoppage of growth) during the labile period with a curve of variation of the effect stretching over the labile period into the one at which wing determination is com-

pleted. The part of the curve to the right means, of course, normal wings; the one covering the labile period, tet wings. Percentage penetrance is thus a function of the location of these curves relative to the time of irreversible determination. The fact that there is no correlation between penetrance and expressivity in the majority of tet wings, although such a correlation does exist in the extreme case of tet-100 (see fig. 25), requires the assumption that the tet effect upon growth as measured by the time of onset in the labile period is larger early in that period and diminishes exponentially with progress in time.

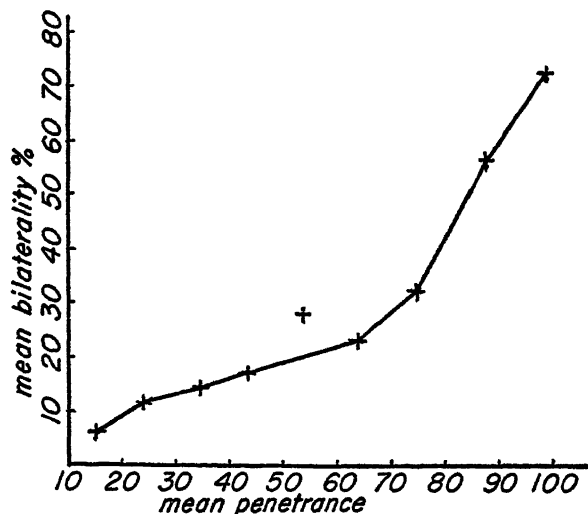


Fig. 33. Correlation between mean penetrance and mean bilaterality in tetralters.

There remains still the problem of asymmetrical vs. symmetrical expression. We showed above that bilaterality is due simply to the effects of independent variation of penetrance on the right and left sides, which means very low probability of bilateral expression when the penetrance is low, and an exponential increase of bilaterality with penetrance. The curves in fig. 28 show this to be true. Theoretically, 100 per cent penetrance should therefore entail 100 per cent bilaterality. But it does not (see curve, fig. 33). Therefore, some other factor is involved beyond independent right-left variation of penetrance and working in the opposite direction. We might conceive of this factor as the amount of growth-inhibiting substance which at the higher levels of penetrance does not increase proportionally and therefore deflects the curve of bilaterality below expectation. We could express this idea also by saying that the need of both discs together for the inductor substance is larger than the available amount in case of highest penetrance.

The phenogenetic situation in tet might be represented by the following diagram (fig. 34), which also illustrates the events in pod, as will be seen below. The abscissa represents time of development with the known stages (here relating to the wing disc) of early development, the time of labile deter-

mination studied by Waddington, Vogt, and others, and the specific differentiation which includes the growth after pupation. Irreversible determination, indicated by the vertical line n , occurs soon after pupation. The upper curve is the normal growth curve of the wing disc. We emphasized above that the tet effect is nothing but stoppage of growth followed by differentiation of what is available at that time. As the tripartite palplike structure is supposed to be

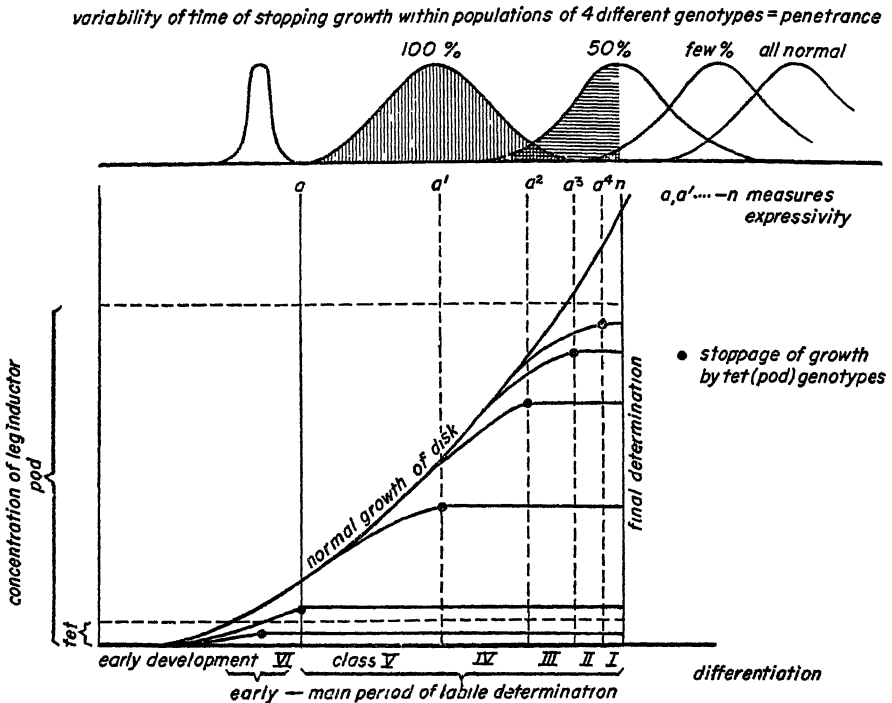


Fig. 34. Diagrammatic representation of phenogenetic explanation of the podoptera effect.

the early structure of all appendages, all grades of tet up to the haltere type are explained if the tet effect consists in stoppage of growth followed by differentiation of what is available at that time. There is no need for further specific inductors. The five other curves, marked with the time of stoppage (black dot) then represent the situation for the different degrees of tet: late stoppage, a short time before final determination of the membranaceous wing, produces class I of the tet effect, early stoppage produces class V. The stage of the disc at the time of stoppage of growth before irreversible determination will be represented in the end product (which, of course, is enlarged by growth after determination). Within the labile period Vogt had found progressive decrease in reactivity of the arista anlage to external factors, which means probably increase of determination, although this determination is not as yet irreversible. In tet it was shown (see p. 138) that expressivity is not proportional to the percentage of penetrance in cultures which have a low degree of pene-

trance, but is well correlated in the special case of tet-100. Expressivity is measured in fig. 34 by the distance of a , a^1 , etc., i.e., the time of growth stoppage, from the time of final determination as indicated in the diagram by the classification below, or by the shape of the anlage reached at the time of stoppage, a logarithmic arrangement of the points of stoppage from left to right, i.e., crowding to the right (see a , a^1 , etc., in fig. 34). We suspect that this is somehow based upon a continuous increase of determination during the labile period, which could be indicated by a logarithmic curve reciprocal to that for expressivity. The relation of this interpretation of expressivity to that of penetrance is shown in the variation curves on top. These represent the variation of the location of the stoppage points in populations with different penetrance, all points to the left of the line of final determination exhibiting the phenotype in question (the shaded areas).

How does pod fit into this scheme? In some respects pod shows features closely parallel to those of tet. It shows a parallel series of wing reduction with similar features of penetrance and expressivity, which means that all this must be explained in a similar way by time of stoppage of growth. But in tet the entire wing behaves as a unit, thus making the series of phenotypes, in a general way a series of developmental stages of the normal wing. In pod, however, the stoppage of growth, as well as the differentiation, occurs in different ways in the four sections of the wing, and only the anterior one forms a leg without tarsus (wingleg). Therefore, in addition to the alteration of growth something else must occur in pod. The factors involved must be of much higher specificity.

In an earlier paper (Goldschmidt, 1945) it was pointed out that Berlese (1909) had assumed, on a comparative morphological basis, that the insect wing consists of four parts arranged in an antero-posterior order. It can hardly be coincidence that the series of pod wings exhibits a comparable subdivision of the wing, of which only the anterior, costal part forms the wingleg. Thus, we are forced to make the following assumptions: (1) The wing anlage has, after a certain stage in development (see below) a basically quadripartite structure. In terms of preliminary determination these four parts are (*a*) the future costal parts, (*b*) the anterior ala, (*c*) the posterior ala, and (*d*) the alula. This preliminary determination is not necessarily morphologically visible at this stage, just as in so many parallel cases known to the experimental embryologist. (2) In addition to the features of growth of the wing disc, discussed for tet, there must be present in pod a leg inductor (see above, the analysis by Vogt). (3) The leg inductor acts only when normal growth of the disc has stopped. (4) The inductor affects only the material determined to become the anterior, costal part of the wing.

If pod and tet are compared on the basis of the above points, there are two possibilities. Either tet has no leg inductor in the wing disc and the specific pod factor produces such an inductor, or both have a leg inductor which in tet has a low concentration, and in pod a high concentration, i.e., the pod factor increases the production of the leg inductor (or increases the reactivity of the anterior wing part to it). It is more probable that both have the inductor,

because under some circumstances tet can form a wingleg. This requires that normal growth of the disc is identical with wing induction, whether a special leg inductor exists or not. These assumptions are also incorporated in fig. 34 by marking the level or concentration of leg inductor which in tet is still below the growth curve of class V (no action upon the growing disc) but which in pod is so high that it can act upon all pod groups below the normals (the normal growth curve).

This interpretation throws light on some further aspects of the problem. It is a fact that class V in tet and class VI in pod may have the same phenotype, namely, a more or less rudimentary palplike appendage, consisting of two or three segments. In addition, class VI in tet may assume the form of a real wingleg, and in pod it might look like a haltere. (This extremely rare condition was not mentioned in the descriptive part. It was observed only twice.) This would mean (see diagram) that in tet a very early stoppage of growth has put the disc within the limits of action of the leg inductor. But in pod at this time (see the dot for class VI in the diagram) the wing disc still represents a unit, i.e., it is for unknown reasons not determined as a quadripartite structure as yet and therefore reacts as a whole. Whether the resulting structure is more palpus or more leglike depends in each case upon the time of stoppage of growth and the amount of growth after determination which is still possible. But there is one rather rare occurrence which is difficult to explain. Occasionally a pod individual shows the tet type of wing reduction in classes IV and V. This means that there was no reaction of the costal part to the leg inductor. For unknown reasons affecting either the concentration of the leg inductor or the reactivity of the substrate, the reaction has failed. But such occasional "errors" are known in all experimental work on induction and do not impair the general explanation.

A second interesting point is the following: We reported that in pod G, class VI does not fall into the series of variation of expressivity but is independent. There might be found a decreasing number of individuals in each of the classes I-III and then a large number in class VI. If we look at the diagram (fig. 34) we see that the variation curves for penetrance (the relation of which to the logarithmic expressivity classes I-V is as discussed above) do not simply extend to the left of class V. There is a hiatus at the beginning of the period of labile determination. In terms of development this would mean that during the period of early development another independent and separate short period of determination must occur, and that the tet and pod reactions which determine stoppage of growth have, in addition to their continuous variation in a population within the long labile period, another, discontinuous variation of the all or none type between the early and late periods of determination. This is probably based upon two different absolute thresholds in the two periods. More than one thermolabile period has been found to exist for a number of mutants (see Goldschmidt, 1937, for references); for others existence of such periods appears probable. The exact meaning of these assumed thresholds in terms of embryonic processes can be found only by embryological experimentation. In the diagram (fig. 34) these facts are indicated by the special curve (on top) for class VI.

A third, interesting problem of determination is presented by the fact that in a number of cases both tet and pod show a tendency to duplication in a very characteristic way (see description above, p. 90). In pod the costal part may be duplicated, i.e., an anterior wingleg or a palp is formed, but the ala behind it has also a second, complete costal region; or in addition to one of the pod phenotypes an independent palplike appendage appears in front of the transformed wing. In tet, however, it is the posterior end of the wing, the third posterior cell, which in class II shows a tendency to mirror-image duplication. There are a number of mutants of *Drosophila* which are known to produce a tendency to duplication of appendages, and duplications of every type of appendage are known as occasional freaks. For our present problem those in which a mutant produces a change in the eye and, in a certain percentage of cases, different degrees of antennal duplication are of special interest. One such mutant, an allele of Deformed, has recently been analyzed by Vogt (1946-1947); another one, an allele of Lobe, is being investigated in our laboratory. In both, the eye is more or less rudimentary and sometimes bulging. It is safe to assume that in these cases mechanical distortions within the eye-antennal disc, which is a common structure in these early stages, makes the antennal anlage separate into two equal parts or induces it to sprout a daughter part which results in the different grades of duplication. A still better parallel is found in the intersexes of *Lymantria* (see Goldschmidt, 1921). In those male intersexes, which still have a more or less normal differentiation of the Herolds organ into valvae and penis, these structures tend to be duplicated to varying degrees, which means that in some way the female determination of the male intersex induces a distortion and subsequent splitting of the anlagen of these organs. When the leg inductor of pod acts upon the costal part of the wing it might create a similar upset, which occasionally results in a splitting of the anlage. No facts which would permit a more detailed statement are available. In the tet wing the situation must be different. Here the change in the posterior cell toward a duplication seems to be connected with events at later stages of differentiation. At present no explanation can be given.

Another type of duplication, however, is found in tet (only very rarely in pod), namely, duplication of the posterior part of the thorax with all its bristles (see figs. 1ef, 2a, in Villee, 1942). It is most probable that the abnormal reduction of wing tissue leaves more room for the expansion of the dorsal mesothoracic portion of the same disc. This may lead to duplication.

One more fact should be discussed, namely, the frequency of occurrence of individuals with all grades of hemithorax, bilateral or unilateral. (They were registered with class VI in pod-G and tet.) Since wing and thoracic anlage are contained in the same mesothoracic disc, a stoppage of growth at a time before determinative separation will affect both, and if the separation of these parts by a determination process is not sudden but gradual, there may result many stages of hemithorax with or without rudiments of knoblike wings. In the diagram this would appear as a stoppage point (not indicated) still further to the left.

There is one problem concerning the leg inductor which should be discussed. It is not customary to introduce phylogenetic ideas into the discussion of problems of experimental embryology. But it can hardly be avoided in a case like the present one. If legs, antennae, mouth parts (proboscis), and wings have a similar morphological basis of development, a palpus-like anlage, and if all contain the active principle called a leg inductor, the reason for it must be their phylogenetic identity. We point to this problem which, discussed earlier by the senior author (1945) in a general way, is now presented in a more special form in the foregoing phenogenetical discussion.

A last problem is presented by the action of the Hairless factor in enhancing some of the pleiotropic podoptera effects without affecting others. In pod and tet stocks containing Hairless, a number of flies are found in which the wing is not fixed at right angles, although it may be blistered and changed to one resembling class II of pod and tet. Furthermore, the legs are highly abnormal, remaining frequently in a developmental condition in regard to growth but not in regard to differentiation. It should be stated first that the fact that class I in pod and tet is represented by a normal wing fixed at right angles because of the absence of a normal wing joint (as can be ascertained by manipulating the wing) requires the assumption that determination proceeds from tip to base, in order to explain that development of the base can be abnormal because of pod action after the rest of the wing is already determined and is developing normally. As far as the action of the mutant H is concerned, this means that this mutant affects developmental processes after the whole wing with its base is determined. It stops some later growth processes, such as growth after final determination of the ala and concrescence of the wing blades, and results in blisters. It also facilitates the spread of a growth-stopping reaction. In pod and tet, rarely more than one leg, if any, is affected, and the abnormalities are never extreme. The leg involved is very frequently the one on the same side on which the wing effect is manifested. This might be interpreted as a slight spreading of the wing-growth inhibiting reaction of pod to the leg discs. Hairless somehow removes the block to further spreading of the effect beyond the wing disc, thus affecting the later development of the legs very strongly. One might say that H diverts the pod action to later stages and to a wider field.

Rapoport has tried to find an embryological interpretation of the podoptera effect in a different way, although his discussion is more generalized. He became especially impressed with the pleiotropic effects upon thorax, scutellum, and bristles. The occasional broadening and expansion of the thorax concomitant with the wing reduction suggests to him that, at a stage when thorax and wing discs are united, a change occurs which makes a part of the presumptive wing disc become incorporated into the thorax. This interpretation does not agree with the facts in tetraltera and podoptera. Nowhere could a correlation be found between the mass of the wing rudiment and that of the thorax. We are inclined to consider the thoracic abnormalities an accommodation to the abnormal spatial relations resulting from the presence of the changed wing disc. In our material this is, moreover, only a rare phenomenon.¹

¹ Addition, November, 1950: A new dominant Pod under investigation seems to bear out

PART III. GENETIC ANALYSIS

The genetic analysis of the pod effect presented difficulties not usually encountered in *Drosophila* work, owing to a number of peculiarities which will become evident in the following analysis.

1. SEGREGATION IN OUTCROSSES

General information on independently segregating pod factors in tet and pod was obtained by outcrosses, which were made with the special Columbia Florida line, a line practically free of pod factors. This method follows the classical procedure for the analysis of multiple factors but is much less simple because of the incomplete penetrance of the character.

A. TETRALTERA

Tet flies from the tet stock kept by mass selection were outcrossed to Florida flies. The penetrance of tet in the broods from which the tet parents came is known. But we saw before (see selection for penetrance) that this line was still genetically mixed, and that a high-penetrance brood might be homozygous for all tet factors or merely a plus variant of a partly heterozygous combination. The F_2 results lumped from many sister broods will therefore not give a reliable quantitative picture of the segregation. In the next chapter will be presented the correct results derived from completely homozygous tet parents. In the present group of crosses, however, some basic facts become clear. The F_2 results are tabulated in table 57. All the F_2 ratios are very low, the highest being less than 3 per cent penetrance. (In one column the number of flies per bottle is reported to show that crowding does not consistently account for the difference.)

If we compare reciprocal crosses we find that the differences between female and male penetrance are greater in the crosses $(Fla \times tet)^2$ than in the reciprocal ones, i.e., $\frac{\text{♀ pen.}}{\text{♂ pen.}}$ in the first = 4.2, 2.7, 5.3, 2.6, averaging 4.0 per cent;

in the second, 2.0, 2.6, averaging 2.4 per cent. As the second group permits females to be homozygous and males hemizygous for a sex-linked factor, the presence of such a factor would account for the difference. In this case higher absolute female penetrance would also be expected in the group where the grandmother was tet. If we take the strictly comparable cases, nos. 4, 5, 7, and 8 in table 57, we find penetrance to be: $(Fla \times tet)^2$ = female, 0.25 per cent; male, 0.5 per cent; $(tet \times Fla)^2$ = female, 0.5 per cent; male, 1.2 per cent. These results indicate that a sex-linked factor is involved, but there is still a considerable difference between the sexes in regard to penetrance in the second group in which the sex-linked recessive is present in both females and males equally. This difference disappears in the succeeding generations, in which tet has higher penetrance. It is probably purely environmental. Where it is observed we call it the sex specific action.

If we take nos. 1 and 2 from table 57, which exhibit relatively high penetrance based upon grandparental constitution, we might draw some initial

TABLE 57
F₂ FROM SELECTED tet × FLORIDA
(Percentage of tet penetrance in parentheses)

No.	Cross	+	tet ♀	tet ♂	Average per bottle	Remarks
1	(Fla × tet) ²	2,165	6 (0.55)	26 (2.3)	244	Grandfather from brood with 70 per cent penetrance
2	(Fla × tet) ²	1,165	5 (0.85)	17 (2.8)	297	Grandfather from brood with 54 per cent penetrance
3	(Fla × tet) ²	4,894	8 (0.32)	41 (1.7)	330	2 pairs per bottle; 15 broods from different F ₁ grandfathers, same line
4	(tet × Fla) ²	659		1 (0.15)	329	2 pairs per bottle
5	(tet × Fla) ²	1,235	3 (0.48)	6 (0.96)	250	2 pairs per bottle
6	(Fla × tet) ²	1,237	1 (0.16)		309	A different series
7	(Fla × tet) ²	2,023	3 (0.3)	8 (0.79)	203	Pair breeding; penetrance of grandparents tet = 37 per cent
8	(tet × Fla) ²	2,156	8 (0.73)	17 (1.6)	218	Pair breeding; penetrance of grandparents tet = 37 per cent
9	(all Fla × tet) ²	11,484	23 (0.4)	90 (1.6)	...	
10	(all tet × Fla) ²	4,050	11 (0.5)	24 (1.2)	...	

conclusions concerning the genetic factors involved. The mean female penetrance is 0.66 per cent, the mean male penetrance is 2.5 per cent. The female penetrance is here based solely upon autosomal recessive factors. Male penetrance is the sum (or product) of autosomal and sex-linked factors. We can make an estimate of the action of the latter from the data on the reciprocal cross where females and males are alike (as discussed previously). Actually, about one-half to three-fourths of the male penetrance is due to the sex-linked factors.

Although the very low F_2 penetrance in this experiment is partly due to genetic heterozygosity of different tet factors in the original ancestor, this cannot account completely for the result if multiple and not multiplicate factors are involved. (The latter would produce ratios 15 : 1 or 63 : 1 multiplied by the percentage of the homozygous line.) Actually, the selection experiments presented above, as well as all the data to follow, prove that penetrance of tet is based upon the recombination of multiple factors. If these had a simple additive effect, and if each one taken singly was able to produce the tet phenotype, a much higher F_2 penetrance would be expected, namely, one-fourth of the penetrance in the homozygous parental line. Penetrance of the order of magnitude found would be possible only if the individual multiple factors have very small individual effects which increase geometrically when they are combined. The facts to be reported will bear out this assumption. Thus, we face a genetic situation which is intermediate between the types of multiple and multiplicate factors in some respects. If the genetic increase of the action of combined factors is not very high, the F_2 percentage of penetrance will not be much above the expectation for multiplicate factors, at least with only a few pairs of individual factors. The actual percentages found point thus toward 2 or 3 pairs of independent autosomal factors with multiplicative, not additive, action.

Extracted F_2 phenotypically normal flies might be free of tet factors, heterozygous for them, or might be nonpenetrants of the different homozygous combinations of tet factors, including the sex-linked ones. Eighteen pairs of tet $\times$ + and 3 pairs of tet $\times$ tet were extracted from F_2 . Of the first group, 6 broods were made from a single F_2 family, 12 more + $\times$ tet were derived from different F_2 broods, as were the three tet $\times$ tet. Table 58 contains the results. There were more F_3 broods phenotypically normal than broods containing tet, even when both parents were tet. Obviously, none of the combinations produced homozygosity for the autosomal or sex-linked factors, except possibly for a factor of very low action. F_4 (the same table) was bred from tet $\times$ +, and the majority of broods contained tet flies. Since, repeatedly, only tet males appeared, the hemizygous tet factor had been selected here.

In F_5 there were still two broods from tet $\times$ + matings without tet flies, but as only 63 flies were obtained, this is not significant. After F_5 no broods without tet flies were obtained, although F_6 was again bred from normal flies. This means that at least one tet factor had become homozygous. Actually, homozygosity for all major factors had been accomplished in F_5 for two lines, as shown by no. 12 (tet $\times$ +) and no. 14 (tet $\times$ tet). Each had a penetrance of

TABLE 58

EXTRACTED $F_3 - F_8$ FROM (tet $\times F_{1a}$)²

No.	Parents	Number and type of crosses	No. of broods		No. of flies in broods		tot		Remarks
			Without tet	With tet	Without tet	With tet	1	6	
1	+ $\times$ tet.....	6 sister broods . . .	3	3	254	364	1	6	1 φ 1 σ , 3 σ , 2 σ tet resp.
2	+ $\times$ tet.....	12 cousin broods....	8	4	922	516	2	4	2 $\times$ 1 φ , 2 $\times$ 2 σ tet
3	tet $\times$ tet.....	3 cousin broods . . .	2	1	301	175	..	1	
4	Total.....	21 broods.....	13	8	1,477	1,055	3	11	

Extracted F_4									
5	$F_3 + \times$ tet.....	2 sister broods . . .	1	1	225	143	13	7	
6	$F_3 + \times$ tet. . . .	5 cousin broods.....	..	5	...	687	5	19	3 $\times$ only σ (16)
7	F_3 tet $\times$ +.....	3 cousin broods.....	1	2	240	185	..	4	

Extracted F_5									
8	$F_4 + \times$ tet.....	6 sister broods . . .	1	5	30	950	32	31	from F_4 6 with many σ (10)
9	$F_4 + \times$ tet. . . .	3 sister broods	..	3	..	476	16	29	from F_4 6 with many σ (+)
10	$F_4 + \times$ tet.....	2 sister broods	..	2	..	403	10	17	from F_4 7
11	$F_4 + \times$ tet. . . .	2 sister broods	..	2	..	179	17	32	from F_4 6
12	F_4 tet $\times$ +.....	2 cousin broods . . .	1	1	33	34	57	60	from F_4 6
13	$F_4 + \times$ father tet...	2 cousin broods . . .	..	2	..	398	9	17	from F_4 6
14	F_4 tet $\times$ tet.....	2 sister broods	..	2	..	23	48	55	from F_4 6
15	F_4 tet $\times$ tet.....	2 sister broods.....	..	2	..	166	40	43	from F_4 6

Extracted F_6									
16	F_5 tet $\times$ +.....	3 sister broods..	..	3	...	383	28	46	from F_5 9
17	F_5 tet $\times$ +. . . .	2 sister broods . . .	..	2	..	195	5	8	from F_5 13
18	F_5 tet $\times$ +.....	1 brood.....	..	1	..	78	15	25	from F_5 8

19	$F_6 + \times \text{tet.}$	2 sister broods.....	..	2	...	93	8	7	from F_6 9
20	$F_6 \text{ tet} \times \text{tet.}$	3 sister broods.	..	3	...	106	7	11	from F_6 9
21	$F_6 \text{ tet} \times \text{tet.}$	1 sister brood.....	..	1	...	25	43	46	from F_6 9
22	$F_6 \text{ tet} \times \text{tet.}$	3 cousin broods.	..	3	...	170	58	85	from F_6 15
23	$F_6 \text{ tet} \times \text{tet.}$	2 sister broods..	..	2	...	232	87	81	from F_6 13
24	$F_6 \text{ tet} \times \text{tet.}$	1 sister brood..	..	1	...	28	33	40	from F_6 13
25	$F_6 \text{ tet} \times \text{tet.}$	F_6 13 $\times$ father..	..	1	...	192	8	18	from F_6 13
26	$F_6 \text{ tet} \times \text{tet.}$	1 brood.....	..	1	...	72	18	11	from F_6 16
27	$F_6 \text{ tet} \times \text{tet.}$	7 sister broods....	..	7	...	604	17	56	from F_6 17
28	$F_6 \text{ tet} \times +$	1 brood.....	..	1	...	137	2	16	from F_6 16
29	$F_6 \text{ tet} \times +$	1 brood.....	..	1	...	151	21	13	from F_6 16

 Extracted F_7

30	$F_6 + \times \text{tet.}$	2 sister broods.....	..	1	...	109	1	1	from F_6 tet $\times +$
31	$F_6 + \times +$	1 cousin brood.....	..	1	...	210	2	2	from F_6 tet $\times +$
32	$F_6 + \times +$	2 sister broods.....	..	1	...	247	1	2	from F_6 tet $\times +$
33	$F_6 \text{ tet} \times \text{tet.}$	1 brood.....	..	1	...	138	26	55	from F_6 tet $\times \text{tet}$
34	$F_6 + \times +$	3 sister broods.....	..	1	...	152	30	52	from F_6 tet $\times \text{tet}$
35	$F_6 \text{ tet} \times \text{tet.}$	1 brood.....	..	3	...	281	40	41	from F_6 tet $\times \text{tet}$
36	$F_6 \text{ tet} \times \text{tet.}$	2 cousin broods. ..	..	1	...	177	15	16	from F_6 tet $\times \text{tet}$
37	$F_6 \text{ tet} \times \text{tet.}$	2 cousin broods.....	..	2	...	196	47	67	from F_6 tet $\times \text{tet}$
38	$F_6 \text{ tet} \times \text{tet.}$	All group 1.....	..	2	...	9	29	31	from F_6 tet $\times \text{tet}$
39	$F_6 \text{ tet} \times \text{tet.}$	All group 2.....	..	4	...	288	55	60	from F_6 tet $\times \text{tet}$
40	$\text{tet} \times \text{tet.} + \times +$	All group 3.....	..	3	...	335	65	128	from F_6 tet $\times \text{tet}$
41	$+ \times +, + \times \text{tet.}$	All group 4.....	..	3	...	686	5	5	from F_6 tet $\times +$
42	$\text{tet} \times \text{tet.}$	No. 42 + parents 21..	..	2	...	9	29	31	from F_6 no. 21 tet $\times \text{tet}$
43	$\text{tet} \times \text{tet.}$		..	3	...	34	72	77	$F_6 + F_7$ high penetr.

 Extracted F_8

44	$\text{tet} \times \text{tet.}$	3 sister broods.....	..	1	...	126	56	70	from F_7 group 2
45	$\text{tet} \times \text{tet.}$	1 brood.....	..	1	...	71	13	40	from F_7 group 2
46	$\text{tet} \times \text{tet.}$		..	1	...	82	26	23	from F_7 group 2
47	$\text{tet} \times \text{tet.}$		..	1	...	60	58	43	from F_7 group 2

86 per cent, which we had found probably to represent maximum homozygous penetrance in this line. Other F_5 matings give from brother-sister, as well as from cousin broods, low penetrance and a normal sex ratio of tet. This means that lower tet factors had been isolated, resulting in production of an average penetrance of 6 per cent. The sex-linked factor is thus not involved. Two autosomals of multiplicative action may be responsible for the effect. In other combinations, 9.2 per cent penetrance in females and 15.8 per cent in males were found, which would mean that, in addition to two homozygous autosomals present in the females and the males, the sex-linked factor is acting in the males. As the latter is probably here present only in half of the males, a male penetrance of about 32 per cent would be expected in a completely homozygous stock. We saw, above, that the X-chromosome factor approximately doubles the autosomal action, and that a male specific action again doubles this, if it is present at all. Thus, the facts agree with the earlier analysis and indicate also that autosomal and sex-linked factors must have a multiplicative action. Further combinations with about 33 per cent penetrance in both sexes might be due to action of three autosomals alone, or to that of two autosomals and one sex-linked factor in both sexes, lacking the usual preponderance of males (called above male specific action).

In F_5 bred from all possible F_5 phenotypes we find again low, high, and intermediate ratios in offspring of matings $\text{tet} \times \text{tet}$ or $\text{tet} \times +$, but only low ratios, with or without sex differences in the offspring of $+ \times +$. The average penetrance for broods with normal sex ratio and low penetrance is 10.5 per cent; for broods with a difference in the sex ratio it is 3.5 per cent in the females and 16.2 per cent in the males. This agrees in the order of magnitude with the expectations derived from F_5 . The difference might be due to selection of two autosomal factors, one of them having lower penetrance. A very high penetrance, 76 per cent, appears twice in broods which have sister broods of low and intermediate penetrance, as expected if the parents were not yet fully homozygous. In both cases both parents were tet. Other ratios, also occurring in sister broods of $\text{tet} \times \text{tet}$, are: 16.7 per cent average in both sexes, and in both sexes an average of 40.5 per cent.

Since we saw that all male effects together (i.e., sex-linked factors plus male specific effects) may quadruplicate that found in the females of the same autosomal constitution, the 16.7 per cent, as well as the 40 per cent average, must be purely autosomal. This probably means that the second belongs to the group with 2, or to one with 3, autosomals, and the first belongs to the group with 1 autosomal factor only.

F_7 selected from different F_5 broods of different ancestry shows again the different groups of ratios. Clearly, the very high penetrance combination of the original P-male has been recovered in F_5 . The offspring of F_5 flies of the brood with 76 per cent penetrance have 87 per cent penetrance in F_7 (and in F_5 62 per cent). A very low penetrance group (1.4 per cent) appears in 2 sister groups and 1 cousin group; penetrance as low as this has been found before only in F_5 . As these matings were derived from a low penetrance F_5 without the sex-linked factor, we must assume that either the autosomal com-

bination with 3-10 per cent penetrance is based on 2 factors which alone have very low penetrance (see the still lower penetrance stock mentioned above) or that a single factor with a slight dominance is involved (an assumption which does not agree with the F_1 facts). Two more groups of penetrance can be clearly discerned. One (no. 39) shows 28.5 per cent penetrance, which is near the 30-33 per cent already discussed. In the other one (no. 40) we have 24.6 per cent penetrance for the females and 48.4 per cent for the males. In view of the unavoidable crudeness of the calculations, the females and males might represent the homozygous group for 2 autosomals and 1 sex-linked factor. The few F_2 broods do not offer new information.

TABLE 59
DEGREES OF PENETRANCE OF TETRALTERA AND ASSUMED
GENETIC BASIS

No.	Percentage of penetrance	Postulated recessive factors	
		Autosomal	Sex-linked
1.....	1	aa	..
2a.....	4	bb	..
2b.....	4		+
3.....	10	aabb	..
4.....	?	cc	..
5a.....	16	aa	+
5b.....	16	aabbcc	..
6.....	33	bb	+
7.....	46	aabb	+
8.....	>65	aabbcc	+

The interpretation of these data has been put into a tentative scheme, in table 59, which agrees roughly with the facts of this experiment as well as with the selection experiment reported earlier (see curve, fig. 14). If we calculate the expectation for F_2 from a completely homozygous grandparent under the assumption made in table 59, a penetrance of approximately 10 per cent is expected. We shall see below that a similar value was found (12 per cent) when this F_2 was made.

B. PODOPTERA G

A comparable test cannot be made with pod-G because of the low penetrance. In an experiment of reasonable size an error larger than the percentages of pod might be found. The difficulty of obtaining proper matings would further complicate the experiment. With a penetrance of 2 per cent, 0.50 per cent would be expected in F_2 for independent and additive autosomals. In a back-cross, assuming a single pod factor, 1 per cent penetrance would be expected. One set of single-pair backcrosses using only pod phenotypes and, for the normal stock, the Florida stock mentioned above, was made in numbers sufficiently great to achieve significance. The result was:

1. (pod $\times$ Fla) $\times$ pod 1,130+, 6 females 4 males pod 0.88 per cent penetr.
2. (Fla $\times$ pod) $\times$ pod 877+, 2 females 5 males pod 0.79 per cent penetr.

This shows that no major sex-linked factor is involved, and that the number of autosomal recessives which produce the pod effect cannot be determined from the results of the backcross. But it can be stated that duplicate factors certainly are excluded.

C. PODOPTERA H

Determination of the number of pod factors in this strain could not be made using the method described above, because of the low penetrance and because results obtained in reciprocal crosses indicated that more than simple multifactorial inheritance was involved.

TABLE 60
INCIDENCE OF PODOPTERA IN RECIPROCAL CROSSES OF pod-H WITH nonpod STOCKS

	Generation	pod-H cytoplasm			nonpod cytoplasm			x ²
		Total flies	pod	Percentage	Total flies	pod	Percentage	
pod-H × Xple.	1	2,441	34	1.39	1,888	2	0.11	19.93
	2	4,194	4	0.09	2,093	5	0.24	1.16
	3	6,722	25	0.37	3,330	18	0.55	1.10
pod-H × 2ple.	1	967	23	2.38	368	2	0.54	4.02
	2	2,885	3	0.11	2,045	3	0.14	0.00+

Reciprocal crosses with two low-pod stocks were made. In the first, pod-H was outcrossed to a stock having a series of recessive mutants located over the greater length of the X chromosome. The second was similar, but in it the markers were in the second chromosome. The parents, for the F₂ and F₃ were chosen at random. The data are summarized in table 60.

The incidence of podoptera in F₁ was significantly higher when the female was from pod-H, but there was no difference in reciprocal crosses in F₂ and F₃. The incidence of podoptera in both F₂ and F₃ was significantly decreased in the first case but not so in the second. It becomes evident, from these data, that other means would be necessary for determination of the number of factors in the pod-H strain.

D. PODOPTERA K

This podoptera stock produces practically no pod phenotypes in F₁ when outcrossed to nonpod stocks (see table 123). Results of a series of F₂ are presented in table 61. It must be kept in mind that the females of this stock contain attached X chromosomes. In no. 2, where the females are not attached X, the values of podoptera have to be doubled, since the character is suppressed in females without an extra Y (see p. 92); but even with this correction all the values obtained are lower than the expected 5 per cent (assuming 20 per cent average penetrance of pod and a single factor responsible for the phenotype). For two interacting factors the expected penetrance in F₂ would be 1.2 per cent, and for three interacting factors 0.3 per cent podoptera would be expected. The values found are in fairly good agreement with the assumption of three interacting podoptera factors.

If the pod effect were based on two independent recessive factors with additive effect, the expected penetrance in F_2 would be 8.75 per cent regardless of the number of factors involved. The substitution experiments (see p. 221) and the F_2 data indicate that the pod factors do not have additive but multiplicative action; therefore conclusions cannot be drawn about the genetic basis of the podoptera effect in pod-K from the F_2 data. The penetrance of the pleiotropic features of pod-K, ro-l, no-l, and pvm was low in the F_2 with dominant markers, and only slightly higher than half of the expected 25 per cent in F_2 of φ pod-K $\times$ $\underline{y}$; bw; e^4 , ro; ey (Patterson stock) and $\underline{y} \times$ pod-K. (In the Patterson cross, rough eyes were not classified.) In the last two cases

TABLE 61
INCIDENCE OF pod-K IN F_2

Mating	Total	No. of pod		Percentage of pod	Percentage of pod plus pleiotropic characters
		φ	σ		
1a. (Canton $\times$ pod-K) ²	2,193	..	..	...	Not classified
1b. (pod-K $\times$ Canton) ²	508	..	2	0.4	Not classified
2a. (Cy/Pm; H/Sb $\times$ pod-K) ²	2,259	..	8	0.4	1.3
2b. pod-K $\times$ Cy/Pm; H/Sb.....	335	2	1	0.9	Not classified
3. (pod-K $\times$ Patt) ²	1,134	4	23	2.4	13.0
4. ($\underline{y} \times$ pod-K) ²	1,158	2	5	0.6	14.1

the difference between the expected and the observed values might be due to lower viability of ro-l, no-l, pvm flies, but the observed decrease in penetrance of these phenotypes should probably again be interpreted to mean that their pleiotropic manifestation is based on the action of more than a single factor. The percentage of phenotypic podoptera is highest in the F_2 with Patterson. This indicates that genetic factors for the phenotypic expression of pod-K, which are lacking in the other stocks, are found in the Patterson stock. It is likely that a combination of these factors with the pod factor in the dominant svr^{Poi} stock accounted for the origin of the high penetrance of podoptera in pod-K, which arose after outcrossing svr^{Poi} to the Patterson stock.

In the F_2 , after outcrossing a pod-K female to Patterson, 27 podoptera flies were obtained. Four of these were females, the rest were males. Cytoplasm and autosomes are the same in the males and females. The difference in manifestation of pod between the two sexes must therefore be due to some factor or factors in the sex chromosomes. (The females are $\underline{XXY}$ and therefore can show the pod-phenotype.) The distribution of the sex chromosomes in the matings pod-K $\times$ Patterson and for comparison in the matings $\underline{y} \times$ pod (no reciprocal cross with Patterson was made at that time) is the following:

pod-K $\times$ Patt		$\underline{y} \times$ pod-K	
φ	σ	φ	σ
P $\underline{X}$ pod Y pod	X Patt Y Patt	$\underline{X} + Y +$	X pod Y pod
F ₁ $\underline{X}$ pod Y Patt	X Patt Y pod	$\underline{X} + Y$ pod	X pod Y +
F ₂ $\underline{X}$ pod Y pod	X Patt Y Patt	$\underline{X} + Y +$	X pod Y pod

From the diagram it can be seen that in F_2 the sex chromosomes are present in the same combinations in which they entered the cross. Therefore, in F_2 , males from the cross $\text{pod-K} \times \text{Patterson}$ carry both sex chromosomes of the Patterson stock; the females, on the other hand, carry the sex chromosomes of the podoptera stock. In the mating $y \times \text{pod}$, the reverse situation holds. In this F_2 , only 7 podoptera flies were obtained, but it does not seem that the difference in the penetrance of podoptera would be found to be very striking in a larger series, for, of the 7 podoptera flies, 2 were females. Since in the pod-K stock females appear with a slightly greater frequency than the males, the absence of females in the cross just discussed cannot be explained.

TABLE 62
INCIDENCE OF PODOPTERA IN CROSSES OF $\text{pod-M}(3)124$ WITH CANTON ISOGENIC

Generation	Mating	Total	Percentage of pod M	Percentage of pod not M	Total percentage of pod
F_2	$M \times M$ or $M \times +$	206	5.3	1.0	6.3
F_2	$+ \times +$	897	...	1.2	1.2
F_3	$\text{pod} \times +$	257	...	1.2	1.2
F_3	$\text{pod} \times \text{pod}$	258	...	10.0	10.0
F_4	$+ \times +$	737	...	8.0	8.0
F_4	$\text{pod} \times +$	657	...	6.2	6.2

E. PODOPTERA $M(3)124$

In F_1 crosses with a number of stocks, $\text{pod-M}(3)124$ acted almost completely as a recessive (table 123). After outcrossing to isogenic Canton, a number of matings of Minute and non-Minute flies were made.

A comparison of F_2 bred from Minute and from normal flies is presented in table 62. The data show that in this cross podoptera may appear without the Minute mutant, but the incidence of podoptera in Minute flies is several times as high as in non-Minute flies. F_3 were bred to determine whether high penetrance of podoptera could reappear in the absence of the Minute. The results of this test are tabulated in table 62. The data show that the incidence of pod may be increased by breeding from pod phenotypes in the absence of the Minute mutant. Comparison with F_2 segregation of podoptera in two other outcrosses (table 63, 6, 7) shows that the Canton stock probably contains an allele of one of the podoptera factors of $\text{pod-M}(3)124$, and that therefore any estimate regarding the podoptera factors based on the segregation with the Canton stock cannot be accurate. Other factors which may increase the error in the calculations are the possible slight dominance of the pod factors and the separate action of factors located in the sex chromosomes. From the F_1 results in table 123, however, it is evident that dominance, if any, of the podoptera factors is negligible. This fact excludes the possibility that the Minute mutant alone is responsible for the pod phenotype. At least one additional recessive factor must be involved. The X chromosome does contain podoptera factors (see, below, section on isolated chromosomes and the sex-linked factors in

tet-100), but the occurrence of approximately equal numbers of males and females in F_2 of the attached X crosses (in which case the females never receive the podoptera X) indicates that the major podoptera effect must be due to factors which are located in the autosomes. The results of the F_2 segregation summarized in table 63 may be used for a very rough calculation of the action of those autosomal factors. Assuming that the incidence of pod in the inbred stock varies from 15 per cent to 25 per cent, and that the Minute mutant itself is indispensable for the appearance of the podoptera phenotype, about 3.3 per cent pod would be expected in F_2 for all Minute flies if a single recessive pod factor is required in addition to the Minute mutant. For the non-Minute

TABLE 63
INCIDENCE OF pod-M(3)124 IN F_2

Mating	Total	No. of pod		Percentage of pod M	Percentage of pod not M
		♀	♂		
1. (pod × Cy/Pm; H/Sb) ² both parents M..	169	4	1	2.9	...
2. (pod × Cy/Pm; H/Sb) ² one parent M....	463	1	..	0.2	...
3. (pod × Cy/Pm; H/Sb) ² both parents not M.....	664	..	..	...	...
4. ($\bar{y}$ × pod) ² both parents not M.	325	..	..	...	...
5. ($\bar{y}$ × pod) ² one or both parents M.....	259	1	3	1.5	...
6. (pod × Fla) ²	1,013	..	..	1.6	0.1
7. (Fla × pod) ²	1,724	..	..	1.8	...

flies the incidence would be zero in case the Minute mutant were absolutely essential, or about 1.7 per cent if the Minute mutant were actually of no special importance. The data in table 63 support the assumption of a single strong pod factor and of indispensability of the Minute mutant. Comparison with table 62 shows that the result of the Canton × pod segregation represents a special case. Pod factors in the Canton stock interact with those of pod M(3)124 to produce a higher incidence of pod flies in F_2 .

The incidence of podoptera in F_2 is too small to permit an accurate estimation of the relative importance of the podoptera factors. It appears, however, that unless podoptera factors are being introduced from the stock to which the pod M(3)124 individual is mated, the Minute mutant is of great importance for the manifestation of the podoptera phenotype, as shown by the fact that in nos. 1, 2, and 5 of table 63 all podoptera flies observed in F_2 had the Minute mutant. Appearance of podoptera in the Canton outcross is most likely due to the presence of pod factors in this stock. It is very unlikely that the Minute factor alone accounts for the appearance of podoptera. If it did, podoptera should be found in a mating of two Minute F_1 individuals in their offspring with two-thirds of the frequency with which it appears in the balanced stock, in which all flies are Minute. But this mating does not produce that result. Therefore, factors other than the Minute must be involved in the production of the podoptera phenotype. Sex-linked podoptera factors are

present and have a decided influence (details under section of isolated chromosomes, p. 220, and tet-100, below. Recessive podoptera factors may be present in the left arm of the third chromosome, the second chromosome, and the fourth chromosome. If several additional factors were present and each of them were needed for the reappearance of podoptera in F_2 , a very much lower percentage of pod would be expected in F_2 than that actually found. Thus, it appears that a few factors (one or two) in conjunction with the Minute produce the podoptera phenotype. The action of groups of closely

TABLE 64
THE SEX-LINKED tet FACTOR
($Patt. = y;bw;e^4 ro;ey$)

No.	Genera- tion	Cross	+	tet		
				♀	♂	
1	F_1	$Patt. \times tet$	65	1	1	
2	F_1	$y\ 5121 \times tet^a$	76	..	1	
3	F_1	$y\ 155 \times tet^a$	160	..	..	
4	F_2	From no. 1, ♂ tet... ..	168	..	12	(4♂ tet are bw)
5	F_2	From no. 2, ♂ tet.. . . .	168	..	15	
6	F_2	From no. 3, ♂ tet.... .	247	..	8	
7	F_3	From no. 5, ♂ tet.... .	100	..	15	(43 ♂ +)
8	F_4	From no. 7, ♂ tet.. . . .	259	3	77	(96 ♂ +)
9	F_5	From no. 8, ♂ tet V....	78	..	39	(18 ♂ +)
10	F_5	Stock y $\times$ ♂ tet F_4	82	..	7	(35 ♂ +)
11	F_5	F_4 ♀ tet $\times$ tet stock.. .	55	..	26	(21 ♂ +)
12	F_6	= no. 10 $\times$ no. 10.... .	242	2	19	(104 ♀ 138 ♂ +)
13	F_6	From no. 9.....	169	..	65	(53 ♂ +)

^a y 5121 and 155 are special laboratory stocks.

linked pod factors rather than single loci cannot be ruled out. Another possibility is the presence of a large number of pod factors, which, however, do not have individual specific effects but are so similar in their action that they may be substituted for one another. In this event, any two or three out of this group of factors would produce podoptera.

F. TET-100 AND THE SEX-LINKED FACTORS

In a preceding section it was reported that the tet stock contains a sex-linked factor which, in collaboration with autosomal factors, produces high penetrance of tet. These effects were evaluated in terms of different factorial combinations (table 59). We saw that the sex-linked factor approximately doubles to quadruples the effect of the autosomals, if we disregard, for the time being, possible effects of the Y chromosome. A study of the sex-linked factor in crosses with attached X chromosomes should allow an evaluation of the action of this factor alone, provided that no dominant tet factors are present. The results, using the highly selected tet line, with more than 60 per cent penetrance and supposedly homozygous, are tabulated in table 64.

F₁ shows a very low effect of the X chromosome from tet, namely, 1.3 per cent only for the males, which agrees with expectation from the data presented above (p. 171). There is also one tet female in the cross with Patterson ($\underline{y}$; $\underline{bw}$; e^4 , ro ; ey^2) which is known to contain weak pod factors (p. 175). In F₂ we expect in the males a combination of autosomal and sex-linked factors, parallel to the one analyzed in the last section, while the females should show only the autosomal effect. (Compare with table 59.) Penetrance in males increased to 12 per cent, but there were no tet females.

According to the data from ordinary F₂ crosses, the purely autosomal action in females in F₂ amounts to 0.6 per cent penetrance. Thus, their absence here may be due to sampling, although it must be kept in mind that all females in the present group have an extra Y chromosome which might act as an inhibitor. F₃ (no. 7) shows clearly that by selection (breeding from tet male) the penetrance of tet in males is increased to 28 per cent. This is exactly parallel to the earlier selection experiment, and as this generation has the foreign Y, an action of a specific tet-Y is excluded. Again, there are no tet females—an indication either that the autosomal action requires a recessive homozygous and a sex-linked factor, or that the Y acts as an inhibitor (see below). In F₄, male penetrance is raised to 43.8 per cent, which is about the same as that found in the original stock. The F₄ females have 1.8 per cent penetrance, which shows, as before, either the very low effect of autosomes without X, or the suppressor effect of the Y, or both (in the experiments reported above, p. 173, *aabb* alone produced about 6 per cent to 10 per cent penetrance).

One of the F₄ tet males was tested by outcrossing to a $\underline{y}$ stock, with the result of 16.6 per cent male penetrance, which is far above the F₁ results, and only possible if, by chance, the $\underline{y}$ stock introduced autosomal tet factors. Also, one of the F₄ tet females was tested with the tet stock and had 55.3 per cent male penetrance in the offspring. In the continued selection line (nos. 9, 13) 68.4 per cent and 55 per cent male penetrance was obtained. In an F₂ of the outcross no. 10 (no. 12) 2 per cent females and 12.1 per cent tet males appeared, agreeing with the previous results.

Thus, we see that male selection for autosomes with a constant male X from tet shows about the same collaboration of factors as revealed in the outcrosses. But the autosomal action alone in the females was far below the figures tabulated above. This can only mean that the extra Y has an inhibiting effect in females. A special chapter will be devoted to the action of the Y chromosome.

In view of the importance of these experiments in the complete interpretation of the podoptera phenomenon, the same experiment was repeated, and simultaneously a similar one was carried out with the line tet-100, which, in the presence of all the autosomal factors, produces 100 per cent penetrance. The F₂ results, together with the controls, are tabulated in table 65.

The penetrance for tet males in F₂ is, as before, around 12 per cent, which agrees with expectations set forth in table 59 and also with the values found in the controls. Purely autosomal female penetrance in the controls is 2 per cent, but is reduced to only 0.4 per cent in presence of the extra Y. The X

chromosome of tet-100, acting alone, is more than six times as powerful as that of selected tet. If combined with the autosomes, it raises the male penetrance considerably. In F_3 bred from an F_2 tet male we have a better chance to increase homozygosity of the autosomal tet factors than in an F_3 from a normal F_2 male. Sixteen F_3 broods from tet fathers showed a male penetrance varying from 0 per cent to 43.3 per cent. Most of the higher values involved the tet-100 X chromosome, but some appeared also with the other tet line. Three out of 12 derived from a tet male showed tet females, namely, 1.6, 2.0,

TABLE 65
 F_2 FROM $y \times$ tet CROSSES

Cross	+		tet		% penetrance	
	♀	♂	♀	♂	♀	♂
($\bar{y}$ 5121 $\times$ tet sel) ²	234	316	2	44	0.9	12.2
($\bar{y}$ 155 $\times$ tet sel) ²	222	199	..	32	...	13.9
($\bar{y}$ 5121 $\times$ tet-100 sel) ²	390	391	3	95	0.8	19.6
($\bar{y}$ 155 $\times$ tet-100 sel) ²	356	308	5	59	1.4	16.1
Σ tet sel ²	456	515	2	76	0.4	12.8
Σ tet-100 ²	746	699	8	154	1.1	18.0
Control F_2 (+ $\times$ tet sel) ²	...	...	..	..	2.0	12.1
Control F_2 (+ $\times$ tet-100) ²	...	...	..	..	6.7	6.2
Control F_1 $\bar{y} \times$ tet sel.....	...	...	..	..	...	1.3
Control F_1 $\bar{y} \times$ tet-100.....	...	...	..	..	...	8.6

6.3 per cent penetrance, but in other broods showing a male penetrance as high as 31 per cent to 43 per cent, which requires at least one, probably two, homozygous autosomals, no tet females appeared.

The same type of selection was made for F_4 . Among 14 F_4 broods, male penetrance ranged from 1.6 to 67.2 per cent, the latter, with an X chromosome from the selected tet stock, being in the range of penetrance found when there was complete autosomal homozygosity. The 3 high-penetrance broods of this group (50, 50, 67.2 per cent) had not a single tet female. Out of 14 broods only 4 produced tet females in rather high percentages (1.3, 7.0, 3.6, 3.3 per cent) which, however, are below expectation for two autosomals. F_5 was bred from tet $\times$ tet to increase autosomal homozygosity. Out of 4 broods with male penetrance of 25–53 per cent, 2 had no tet females, the other 2 had 4.7 and 2.9 per cent, respectively.

Thus, it may be regarded as certain that the Y chromosome in tet females reduces penetrance. Decisive data covering this point will be presented in a special section. Otherwise, the contents of this section agree with the evaluation of the action of the different tet factors presented above.

The contribution which the tet-100 stock makes to the analysis will now be presented in more detail. This stock was extracted in F_5 from a cross of M(3) 124 and selected tet, one of the crosses which will be studied below in the chapter on Minutes. Because the sex-linked tet action in tet-100 is much higher

than in selected tet and is responsible for the complete penetrance of tet, it follows that, either the Minute stock contributed an X chromosome with a potent tet factor to the autosomal tet combination, or the sex-linked factor of the M stock combined with that of tet by crossing over. The following facts agree more closely with the latter alternative. (1) Sister broods of the first tet-100 brood (i.e., with 100 per cent penetrance) showed either a normal sex ratio or one with a low female and a high male ratio. In their offspring similar ratios appeared. To explain this we must assume that the parental generation of the first tet-100 brood also contained X chromosomes without the sex-linked factor. (2) In the generations preceding the appearance of tet-100, ratios typical of the selected tet line were obtained, i.e., tet penetrance of 50-80 per cent. This shows that the homozygous autosomals as well as a sex-linked tet factor had been extracted from the original cross. The latter, however, might be the original one from tet or a new one from M(3)124. (3) In F₄, 4 broods derived from the same parental F₃ brood gave ratios which were never encountered elsewhere and must contain the clue to the origin of tet-100, which was started as grandchildren of one of these F₄ broods. The data for the 4 cousin broods are:

a.	52 females +	10 females tet	6 males +	52 males tet
b.	59 females +	3 females tet	10 males +	57 males tet
a + b.	111 females +	13 females tet	16 males +	109 males tet
c.	17 females +	11 females tet	7 males +	15 males tet
d.	50 females +	37 females tet	15 males +	47 males tet
c + d.	67 females +	48 females tet	22 males +	62 males tet

This looks clearly like a crisscross inheritance with few crossover individuals added in *a* and *b* and with a larger crossing over value in *c* and *d*. The following explanation seems to be the most probable one, because it agrees with the different results in later generations, which we just mentioned. We assume homozygosity for all tet autosomals and call the sex-linked recessive tet factor of tet, *t*; and that of M(3)124, *m*. If the mother had received one X chromosome from each parent she would have had the chromosomes *tM* and *Tm*; the father might have had either one of these sex chromosomes. If the father had the normal chromosome, i.e., *TM*, the cross was a crisscross with a chance for crossing over of *M*, i.e., *TtMm* × *TM*. The female offspring would be largely *TTMm* or *TtMM* in equal numbers, and crossovers *TTMM* and *TtMm* are added to this. The males would be *Tm* and *tM*, and the crossovers *TM* and *tm*.

We know that male penetrance for *aabbccet* is about 80 per cent. We know also that *m* has a greater action than *t* (see below), and therefore we may assume that *aabbccmt* has 100 per cent penetrance. Therefore, we expect more than 80 per cent tet males and less than 20 per cent normals among the noncrossovers. Crossovers add equal numbers to both groups and thus quickly increase the percentage of normals with increase in crossover value. The low percentage of normal males actually found in the *a* + *b* results above (almost 13 per cent) would be expected for a crossover value of approximately 10 per cent if all *TM* males were normal. Actually, a small percentage will be tet on

the basis of autosomal action alone. The necessary small correction does not materially change the results of this rough calculation. The number (approximately 11 per cent) of tet females is clearly due to the effect of the autosomals alone and occurs in the expected order of magnitude. The similarity of the size of this group to that of the crossover group in the males is obviously only a coincidence. The calculation is not reliable in detail; the homozygous penetrance varies, roughly, from 60 per cent to 90 per cent, and our calculations were based on a value of 80 per cent. But in a general way it suggests an origin of the tet-100 X chromosome by crossover combination of m and t.

Genetic constitutions of the F_3 flies, different from the case just discussed, are possible. One is $TtMm \times Tm$, i.e., the mother heterozygous and the father carrying an X from M. Half of the noncrossover daughters would have only the autosomal effect, the other half a combination of autosomal plus m effects. The latter combination is of unknown penetrance, but is above 80 per cent in the males. Since females in inbred stocks always show a lower penetrance, the factorial combination aabbccm may, in females, also produce a penetrance lower than that of the males. This is estimated as being about 80 per cent. The crossovers would add equal numbers to both classes. Thus, the expectation for females would be around 54 per cent +, 46 per cent tet. For males the result would be, assuming, as before, a more than 80 per cent penetrance of aabbccm, and 80 per cent if only t is present (see table 59), about 86 per cent tet and 14 per cent +; if aabbccm had only 80 per cent penetrance the result would be 77 per cent tet and 23 per cent +. The values recorded above for the broods c + d—58 per cent females +, 42 per cent females tet, 26 per cent males +, 74 per cent males tet—agrees with this expectation. This interpretation accounts for all types of combinations after F_4 that have been found, including those without sex-linked recessives or those with homozygous tm, the tet-100 combination.

The following experiments permit verification of the preceding analysis:

1. Female $\bar{X} \times$ tet-100, which reveals the penetrance of the X chromosome in the absence of autosomal dominant tet factors: 143 females +, 128 males +, 14 males tet = 8.6 per cent pen. as we found 1.3 per cent penetrance in males with t only, m increases the incidence of tet more than 6 times.
2. Tet-100 $\times$ different marker stocks: 205 females +, 206 males +, 15 males tet = 6.8 per cent pen., which agrees with no. 1.
3. F_2 from no. 1: 422 females +, 370 males +, 106 males tet = 22 per cent pen.

On the basis of the above discussion, about 19 per cent penetrance was expected.

2. LOCALIZATION

A. LOCALIZATION TO CHROMOSOMES BY MARKER CROSSES

a. *Tetraltera*

Villee's work (1942) had shown that standard localization tests with dominant markers failed to give clear results in tetraltera. In F_2 of the cross S/Cy; D/Sb $\times$ tet the penetrance of tet was 0.26 per cent, i.e., much less than that

found in outcrosses with selected tet stock used in our experiments. (Actually, tet at that time had a much lower penetrance than it has in the present selected stock.) In two series of backcrosses of tet $\times$ heterozygous tet the penetrance was 4.6 per cent and 1.5 per cent, respectively. In the backcross with Cy/tet; D, tet. out of 1,549 flies 59 were tet with no dominant markers. 1 was tet Cy, and 11 were tet D. In backcrosses with S/tet; Sb⁺/tet, out of 5,581 flies 59 were tet + and 43 were tet S. If the markers S/+; Sb Mé/+ were used, F₁ contained some tet flies, and in the backcross to tet females there were, among 1,500 flies: 2 females tet +, 1 female and 4 males S tet, 2 females and 3 males Sb Mé tet, and 1 female and 8 males S; Sb Mé tet. From these data it was concluded that tet was a monofactorial recessive situated in the third chromosome (the second backcross) and that Cy and Mé act as dominant enhancers in the heterozygote. The fourth chromosome was excluded by experiments with fourth-chromosome markers. Two mutants which appeared in the third chromosome of the tet stock seemed to permit an exact localization in that chromosome. In the light of our present knowledge this localization was in error, because the a priori assumption was made that tet is located in the third chromosome. The two mutants were an allele of ebony (70.7) and one of glass (63.3). The backcross est gl¹ tet/+ $\times$ est gl¹ tet resulted in (among 9,455 flies):

5 females	33 males tet +
65 females	107 males e st gl ¹ tet
8 females	10 males gl ¹ tet
2 females	4 males e st tet

These numbers were used for a direct calculation of crossing over e-tet and gl-tet. In making the calculation the following facts were overlooked: (1) a sex-linked condition was clearly present, as the sex ratio shows, and (2) the tet + individuals were not necessarily crossovers. If tet factors were present, both in second and third chromosomes (in addition to the first), tet + could be based upon the homozygous second-chromosome factor, and tet e gl upon either the third-chromosome factor alone or third- and second-chromosome factors combined, with a considerable increase of penetrance in the presence of both. Thus, the calculation of the locus from this experiment is erroneous in the light of improved knowledge. If no second-chromosome locus was involved, the calculation should have been made from the females alone; and if both second and third chromosomes were involved, the conclusion must be that the third-chromosome locus seems to be nearer to gl than to e. Therefore, this method was inadequate and was not repeated.

Experiments performed years later gave the results tabulated in table 66. Tests made with fourth-chromosome markers showed that as many tet flies appeared when the fourth chromosome was replaced as there were in the presence of the tet fourth chromosome; we may therefore disregard the fourth chromosome. The table shows for tet that it combines with all markers and their recombinations. In nos. 1 and 5 the females (in parenthesis) have attached X chromosomes and, therefore, their appearance is based upon autosomal tet factors alone, whereas the males can show sex-linked as well as auto-

somal factors. In females, tet is found combined with Sb, H Sb, Pm, Sb and Pm H Sb. In no. 3, both females and males may have a tet X, and therefore its share in the result can only be estimated from the total penetrance, which here is actually so high as to require autosomal action also. About half of the flies carry autosomal markers. Since recessive autosomal factors must be involved in the production of tet, and since no major fourth-chromosome factor was present, this means that the marker stocks (or some of them) contain pod factors themselves, as was subsequently proved (see below).

TABLE 66-

No.	F ₂ cross	Normal	tet (pod) with markers						
			+	Cy	Pm	Cy Pm	H	Sb	HsB
1	(y Cy/Pm H/Sb × tet) ²	781	3(1) ^a	..	4	...	1	1(1)	4(1)
2	(y Cy/Pm H/Sb × pod) ² ..	850	2(2)	..	..	...	...	3(1)	1
3	(ClB Cy/Pm H/Sb × tet) ² × F ₁ no. 1.....	1,522	8(5)	..	2	4(3)	2(1)	6(4)	2
4	ClB Cy/Pm H/Sb × pod) ² × F ₁ no. 2.....	759	2(2)	..	..	...	3(3)	...	...
5	F ₁ no. 1 × F ₁ no. 3...	387	...	..	..	...	1	1	...
6	F ₁ no. 2 × F ₁ no. 4 ..	1,158	...	..	..	...	...	1(1)	...
7	All tet.....	2,690	11	..	6	4	4	8	6
8	All pod.....	2,767	4	..	..	...	3	4	1

^a 3(1) means 3 tet flies, 1 of which is a ♀, etc.

b. *Podoptera G*

Corresponding tests were made with pod-G. Table 67 contains the results. The fourth chromosome was studied separately and found to combine freely with pod. In the 3 backcrosses nos. 2-4, 12 pod were found with both second chromosomes from pod, 12 with only the third chromosome from pod, and 11 with neither pod second or third chromosomes in homozygous condition. Eight pod flies carried both second and both third chromosomes from pod. The first two results suggest that there are pod factors in both the second and third chromosomes. The group without second or third homozygous pod chromosomes requires either pod factors in the first and fourth chromosomes or pod factors present in a marker chromosome. It is remarkable that 23 out of 27 pod with markers are heterozygous for S, which might mean that S acts as a dominance modifier, or that the S chromosome contains a pod factor. In all crosses taken together, the largest number of pod flies are in the + class, indicating that the presence of both the second and third chromosomes from pod is the most favorable combination for penetrance. The next largest class is the S class, with or without D or Sb, in agreement with the previous statement.

Pod crosses with other dominant markers are also found in table 66. The situation in regard to sex-linked factors is the same as described here for tet. But we know that the pod X has very little influence upon penetrance. Again,

in nos. 2 and 6 the females are $\underline{XY}$. Pod females are found in combination with Sb, Pm H Sb, and Pm Sb. The explanation is the same as presented for tet. We shall return to the problem of the dominant markers in a later chapter.

c. Podoptera H

Localization with recessive markers: The low incidence of podoptera in F_2 and F_3 after outcrossing to recessive markers indicated that the penetrance in pod-H was dependent on several factors. To test this, pod-H was crossed

F_2 WITH DIFFERENT MARKERS

tet (pod) with markers

Cy Pm	Cy Sb	Pm H	Pm Sb	Pm H Sb	Cy H Sb	Cy Pm H Sb	Cy Pm H	Cy Pm Sb
..	..	1	2	1(1)	...	...	...	..
..	..	...	...	1(1)	...	...	...	..
			Cy H					
4	..	3(2)	2(1)	5(3)	1(1)	2(1)	2(1)	..
..	..	...	..	...	..	...	...	..
..	..	...	1(1)	1	...	...	...	1
..	..	...	1(1)	1(1)	...	1	..	..
4	..	4	5	7	1	2	2	1
..	..	...	1	2	...	1	...	..

to a stock with recessive markers in the 3 autosomes: brown (bw) in the second, ebony and rough (e^4 , ro) in the third, and shaven (sv) in the fourth. Table 68 presents a summary of the data. Only 113 podoptera flies (with various combinations of the markers) were observed among a total of 13,348 F_2 flies. In series I they are arranged with respect to segregation of the third chromosome markers e^4 , ro, and pod, disregarding the second and fourth chromosomes. In series II the same data are arranged to show segregation of pod and bw, and in series III, sv and podoptera. Obviously, the third chromosome does not contain the principal pod factor, for there are as many podoptera e^4 , ro recombinants as podoptera with no markers, and there are more with than without e^4 , ro. However, these data do not indicate whether the principal factor is in the second or the fourth chromosome, for there were 14 pod brown recombinations and 15 pod shaven. In other words, if one mutant was in the second chromosome, podoptera shaven flies would be present; if another was in the fourth, pod would recombine with brown, but no podoptera, brown, shaven flies should be present. However, 3 flies were podoptera, brown, shaven. Conclusions in favor of either a single-principal factor or two factors of equal strength cannot be made, because of this conflicting evidence. Evidence that the fourth chromosome does not have a strong podoptera factor is presented below.

Localization with dominant markers: Reciprocal crosses were made with a stock containing dominant markers and crossing-over suppressors in all

but the fourth chromosome, e.g., ClB for the first; Cy and Pm for the second; H and Sb for the third. By appropriate crosses the podoptera chromosomes were replaced in different combinations by those containing the dominant markers. The combined data for F_3 (neglecting the X and Y for the present), given in table 69, are arranged in table 70 to test the effect of replacing the second chromosome and the third chromosome.

TABLE 67
TESTING pod-G WITH DOMINANT MARKERS

Cross	not pod	+		S		D		S D		S Sb		D Sb		Sb	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1. (pod-G × S/Cy;D/Sb) ²	13,009	7	5	..	2	1	..	..	..	..	1	..	2	..	..
2. pod-G × F ₁ S/pod; Sb/ pod.....	4,151	2	2	5	1	..	..	..	..	6	1	..	..	1	3
3. pod-G × F ₁ S/pod; D pod.....	943	2	2	3	..	..	..	2	2	..	..	..	..	..	..
4. F ₂ pod D × S/pod; Sb/ pod.....	416	..	..	3	..	..	..	..	..	..	..	..	..	..	..

TABLE 68
Pod-H MARKER RECOMBINANTS IN F₂ AFTER OUTCROSSING pod TO
A STOCK CONTAINING MARKERS IN THE SECOND, THIRD AND
FOURTH CHROMOSOMES

Series	Chromosomes	pod
I	With e ⁴ , ro.....	48
	Without e ⁴ , ro.....	17
	No markers.....	48
II	With bw.....	14
	Without bw.....	51
	No markers.....	48
III	With sv.....	15
	Without sv.....	50
	No markers.....	48

In the first case, the incidence is 2.57 per cent for pod second, and 0.10 per cent for the second replaced. In the second case the incidence is 0.79 per cent for pod third chromosome and 3.22 per cent for pod third replaced. It may be concluded from these data that the principal factor is in the second chromosome, although a low incidence of podoptera is found even if the second chromosome is replaced. Replacement of the third chromosome results in an increased incidence of podoptera, which may be interpreted as either inhibiting pod factors in the pod third chromosome or enhancing pod effects of the H and Sb chromosomes, or pod action of these dominants. (See below, the chapter on dominants.) The low incidence of pod when the second chromosome is

TABLE 69
INCIDENCE OF PODOPTERA II COMBINED WITH DOMINANT MARKERS

	Sex	+			Pm			Cy			Cy Pm			Total		
		Total	pod	Percentage	Total	pod	Percentage	Total	pod	Percentage	Total	pod	Percentage	Total	pod	Percentage
+	♀	1,230	15	1.22	782	7	0.90	727	4	0.55	142	.		2,881	26	0.90
	♂	1,072	15	1.40	613	5	0.82	603	..		663	..	..	2,951	20	0.68
Sb	♀	503	15	2.98	515	18	3.50	371	3	0.81	243	..		1,632	36	2.21
	♂	555	9	1.62	415	11	2.65	448	2	0.45	258	..		1,676	22	1.31
H	♀	367	6	1.61	376	6	1.60	402	2	0.50	203	1	0.49	1,318	15	1.11
	♂	321	11	3.43	204	4	1.36	503	2	0.40	194	..		1,312	17	1.29
H Sb	♀	502	39	7.77	167	4	2.40	181	..	..	215	1	0.47	1,065	41	4.13
	♂	433	18	4.13	150	1	0.66	132	..		180	..		895	19	2.12
Total	♀	2,602	75	2.88	1,840	35	1.90	1,681	9	0.51	803	2	0.25	6,926	121	1.91
	♂	2,381	53	2.23	1,472	21	1.43	1,686	4	0.24	1,295	..		6,834	78	1.14
Σ ♀ ♂		4,983	128	2.57	3,312	56	1.69	3,367	13	0.39	2,098	2	0.10	13,760	199	1.45

replaced may be due to pod factors in the first and fourth chromosomes, or to podoptera factors in the marker chromosomes. These data are arranged in table 71 so as to compare one variable at a time. From this arrangement it becomes apparent that: (1) the strongest factor is in the second chromosome; (2) both H and Sb enhance the expression of the second chromosome (or are

TABLE 70

EFFECT OF REPLACING (a) THE pod-H SECOND CHROMOSOME AND (b) THE pod THIRD CHROMOSOME BY DOMINANT MARKERS

a				b			
2d chromosome	Total flies	pod	Percentage	3d chromosome	Total flies	pod	Percentage
pod/pod.....	4,983	128	2.57	pod/pod.....	5,832	46	0.79
pod/Pm.....	3,312	56	1.69	pod/H....	2,660	32	1.20
pod/Cy....	3,367	13	0.39	pod/Sb.....	3,308	58	1.75
Cy/Pm.....	2,098	2	0.10	H/Sb.....	1,960	63	3.22

TABLE 71

INCIDENCE OF PODOPTERA IN FLIES WITH DIFFERENT COMBINATIONS OF pod-H AND DOMINANT MARKED SECOND AND THIRD CHROMOSOMES

2d and 3d chromosomes	Total	pod	Percentage
2d and 3d chromosomes pod.....	2,302	30	1.30
2d and 3d chromosomes replaced.....	395	1	0.25
2d pod; 3d replaced.....	935	57	6.10
2d pod; 3d heterozygous.....	1,746	41	2.35
2d heterozygous; 3d replaced.....	630	5	0.80
2d heterozygous; 3d heterozygous.....	3,324	48	1.44
2d heterozygous; 3d pod.....	2,725	16	0.59
2d replaced; 3d pod.....	805	..	
2d replaced; 3d heterozygous.....	898	1	0.11

themselves pod factors), but that their effect when together is more than when either is heterozygous; (3) H and Sb act as dominance modifiers when the second chromosome is heterozygous. But these data also indicate that one or more factors may be present in either the first or the fourth chromosomes which give the podoptera effect.

d. Podoptera K

Pod-K was crossed to the Cy/Pm; H/Sb marker stock and a series of reciprocal backcrosses was made. In all the backcrosses to the female of the dominant marker stock only a single podoptera fly (female) was obtained (table 72). The most likely explanation of the appearance of this individual is the assumption

that a podoptera factor was introduced from the dominant marker stock, especially because this fly did not carry both second chromosomes from pod-K and was not X. It is also possible that the combination of H and Sb in the third chromosome has a dominance-enhancing action for the pod factor of pod-K. (See discussion below in the chapter on dominance.) Podoptera segregates with

TABLE 72

RECIPROCAL BACKCROSSES OF pod-K WITH THE DOMINANT MARKER STOCK (Cy/Pm; H/Sb)

Cross	not pod	pod													
		+		Cy		Pm		H		Sb		Pm H		Pm H Sb	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Cy/Pm; H/Sb × F ₁ Pm/ pod; H/pod.....	302	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cy/Pm; H/Sb × F ₁ Cy/ pod; H/pod.....	496	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cy/Pm; H/Sb × F ₁ Pm/ pod; Sb/pod.....	491	..	..	..	..	..	..	..	..	..	..	..	..	1	..
Cy/Pm; H/Sb × F ₁ Cy/ pod; Sb/pod.....	1,295	..	..	..	..	..	..	..	..	..	..	..	..	..	..
pod × F ₁ Pm/pod; H/pod.....	590	16	13	..	..	1	..	3	..	..	..	1	..	..	..
pod × F ₁ Cy/pod; H/pod.....	1,399	28	32	..	1	..	..	1	..	..	..	..	..	..	..
pod × F ₁ Pm/pod; Sb/pod.....	927	25	27	..	..	1	..	..	8	6	..	..	..	..	..
pod × F ₁ Cy/pod; Sb/pod.....	822	21	12	..	1	..	..	..	3	5	..	..	..	..	..

TABLE 73

INCIDENCE OF pod-K IN F₂ WITH THE DOMINANT MARKER STOCK

Cross	not pod	pod											
		+		Cy		Pm		H		Sb		H Sb	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
(Cy/Pm; H/Sb × pod) ²	2,230	..	4	..	1	..	..	..	2	..	..	..	1
(pod × Cy/Pm; H/Sb) ²	332	1	1	..	..	..	..	..	..	1	..	..	..

an average frequency of 7.5 per cent in the backcrosses to the pod-K stock. Of the pod flies obtained (205) only 4 contained one second chromosome of the dominant marker stock. In the majority of cases (174) podoptera was found without any of the dominant markers, but in 26 flies H or Sb appeared together with the pod phenotype. In F₂ with the dominant marker stock (table 73) the importance of the second chromosome from pod-K is again evident.

The series of backcrosses places the major podoptera factor of pod-K in the second chromosome. The ro-l, no-l, pvm complex, which is most likely a pleiotropic effect of the podoptera factor in the second chromosome, segregates like a single second-chromosomal recessive with almost 100 per cent penetrance

TABLE 74
 RECIPROCAL BACKCROSSES OF pod-M(3)124 WITH THE DOMINANT MARKER STOCK

Mating	not pod	pod							
		M	Cy M	Pm M	Sb M	H M	Pm	H	Sb
Cy/Pm; H/Sb × F ₁ pod/markers	3, 217	1	..	..	.	..	..	..	
pod (phenot. +) × F ₁ pod/markers.	909	5	..		..	..	..	..	1 Cy Pm H M
pod (phenot. pod) × F ₁ Pm; M/Sb .	579	25	..	2	5	..	.	..	2 ClB Cy Pm Sb
pod (phenot. pod) × F ₁ Pm; M/H	598	20	..	1	.	3	..	2	1 Cy H M-like, 1 Cy Pm H
pod (phenot. pod) × F ₁ Pm; Sb .	549	30	..	..	12	.	..	..	M-like
pod (phenot. pod) × F ₁ Pm; H ..	178	3	..	..	..	1	.	..	1 Pm M Sb
pod (phenot. pod) × F ₁ Cy; M/H.	337	17	..	..	..	1	.	2	
pod (phenot. pod) × F ₁ Cy; H..	412	15	..	..	..	..		..	
pod (phenot. pod) × F ₁ Cy; Sb...	305	18	..	..	8	..	.	..	1 M Cy Sb
pod (phenot. pod) × F ₁ Cy; M/Sb	227	7	.	..	5	..	..	.	1 M Cy Sb

and somewhat lower viability than normal in the males and in attached X females. Rarely is a male obtained without rough eyes when the second chromosome from pod-K is present in homozygous condition. This means that penetrance of rough eye after outcrossing is occasionally not 100 per cent. The podoptera phenotype probably needs one additional factor for its expression. The experiments with substitution lines to be presented in the next section indicate that no other major factor is involved.

e. Podoptera M(3)124

The localization of pod-M(3)124 factors was determined in a series of backcrosses (table 74). These data give some information on the following points: (1) In backcrosses to Cy/Pm; H/Sb very few podoptera flies reappeared, although one-half of all flies are Minute in any of the crosses in which a Minute male is used. (2) In the backcross to pod-M(3)124, podoptera was obtained in fair numbers only when phenotypically podoptera females were used as backcross females. If a phenotypically normal female from pod-M(3)124 was used, very few pod flies were obtained. The majority of the podoptera flies which segregated carried the Minute mutant. The other third chromosome may be substituted from the marker stock. However, there are higher numbers of podoptera with the Minute balancer, In(3), than with either one of the third-chromosome markers (M H or M Sb). In a few cases, podoptera appeared with one second chromosome substituted from the marker stock. None of these individuals showed an expressivity higher than type III.

The two ClB, Cy Pm Sb podoptera females in table 74 are thought not to be comparable to the other flies obtained in the backcrosses. The appearance of two such individuals in the offspring of one mating would indicate that a low penetrance podoptera factor was introduced from the dominant marker stock in this mating. The Minute-like Cy/Pm; H podoptera individual as well as its Minute-like Cy H sibling also represent a special case. Therefore, the complete substitution of the second chromosome of pod-M(3)124 by dominant markers has no significance in the present discussion of segregation of podoptera with dominant markers. In all other instances only one second chromosome can be substituted from the dominant marker stock if the podoptera phenotype is to manifest itself. This fact indicates that an important factor is present in the second chromosome.

In rare cases pod segregates without the Minute. Whenever this happened, both second chromosomes from the podoptera stock and the Minute balancer chromosome were present in the phenotypically pod individual.

Some idea of the importance of the X chromosome may be obtained from the backcrosses to the pod female shown in table 74. All males are hemizygous for the podoptera X chromosome, the females are heterozygous $X^{\text{pod}}/X^{\text{marker}}$. If important recessive pod factors are present in the X chromosome, the penetrance of pod in the females should be appreciably lower than in the males. Table 74 shows that this is not the case; only a slight difference is found. Although the X chromosome does contain pod factors (see isolated chromosomes, p. 220), its importance in the production of the pod phenotype is not

great. Thus, it appears that in the production of the pod phenotype in pod-M(3)124 the Minute chromosome has the strongest action, the Minute balancer is of some importance, the second chromosome bears a recessive factor of moderately strong pod action, and the X has some pod effect, which, however, is slight if compared to that of the other chromosomes.

B. LOCALIZATION BY REPLACEMENT OF CHROMOSOMES

The following method was used to determine the pod action of individual chromosomes and of their combinations for pod-G, pod-K and pod-M(3)124: (1) $\underline{y}$; Cy/Pm; H/Sb, In(3)R Mo, sr was crossed with pod and F_2 made from complementary dominants, e.g., Cy H $\times$ Pm Sb. This permits the extraction of flies homozygous for the pod second, third, or second and third chromosomes. The females always have the foreign first chromosomes, the males always the pod-X. All females have an extra Y chromosome, and this alternates with the Y in the males so that in odd-numbered generations the females carry the pod Y, and the males carry the pod Y in all even-numbered generations. (2) In order to introduce the homozygous first chromosome of pod into similar combinations, F_1 was made from ClB; Cy/Pm; H/Sb, In(3)R Mo, sr $\times$ pod. It must be added here that the pod-X chromosome is marked by the two position effects y and w (from inversions) which do not cross over in the presence of the larger inversion in ClB. F_1 Bar females were crossed to F_1 males of a mating $\underline{y}$; Cy/Pm; H/Sb $\times$ pod-G (or another podoptera stock). The desired autosomal combinations appear in the resulting offspring, but as the X chromosome of pod-G contains white, the presence or absence of Pm cannot be determined. Therefore, females with the appropriate autosomal constitution but with ClB/X^{pod} were mated to a stock with crossing-over suppressors and sc^8 and $\underline{y}$; Cy/Pm; Sb, In(3R)C/DCX (H. J. Muller's MI stock). In F_2 of this mating the podoptera X was selected in homozygous condition, together with Cy/Pm or H/Sb, or with all four dominants. In all these combinations the females do not have an extra Y, and the male Y is derived from the marker stock. (3) F_1 females from no. 1 were crossed to males of the F_1 no. 2. This permitted, in both sexes, the combination of the chosen autosomes without the pod-X. The Y chromosomes in both sexes were here derived from pod.

In the description of the combinations the pod chromosomes are indicated, e.g., X2 means that only the first and second chromosomes are pod chromosomes, the third and the Y are replaced. When females and males are different, as in attached X lines, they are marked separately. The fourth chromosome can be checked only per exclusionem, because no marker stock with an additional fourth chromosome dominant was viable. As controls, all marker stocks were tested for the occurrence of pod. The results are included in table 75. The isolated lines were bred by single-pair brother $\times$ sister matings. In order not to lose the extracted lines, mass cultures were made. The data on these were kept separate because in mass cultures a different penetrance is found. They are therefore not included in the tables. The results, however, were strictly parallel to those of single-pair broods.

a. Podoptera G

In table 75 the results for pod-G are presented. It must be kept in mind that: (1) If the pod-X chromosome is isolated, the dominant markers Cy/Pm; H/Sb are simultaneously present. (2) If the second chromosome is isolated, II/Sb are present, and Cy/Pm if the third chromosome is isolated. We know (see also controls 13, 14) that these dominant stocks have a podoptera effect of their own. In addition, we shall show below (p. 249) that they have a pseudoallelic effect with pod in other chromosomes. (3) In the presence of attached X in

TABLE 75
ISOLATION OF CHROMOSOMES OF pod-G

(Numbers and Y in second column indicate the pod chromosomes present. $\bar{X}$ = foreign attached X chromosomes.)

No.	Chromosomes		+	pod ♀	pod ♂	pen ♀	pen ♂
1	♀ $\bar{X}$	♂ 1.....	885	7	5	1.5	1.1
2	♀ $\bar{Y}$	♂ Y^a	1,906	10	6	1.0	0.6
3	♀ $\bar{X}2$	♂ 2.....	2,566	51	25	3.9	1.9
4	♀ $\bar{X}3$	♂ 3.....	2,569	13	23	1.0	1.8
5	♀ $\bar{X}2$	♂ 1,2.....	2,439	31	42	2.5	3.3
6	♀ $\bar{X}3$	♂ 1,3.....	2,525	11	33	0.8	2.6
7	♀ $\bar{X}2,3$	♂ 2,3.....	1,051	12	10	2.2	1.9
8	♀ 1	♂ 1.....	1,866	4	3	0.4	0.3
9	♀ 1,2	♂ 1,2.....	2,450	41	10	3.3	0.8
10	♀ 1,3	♂ 1,3.....	1,951	18	14	1.8	1.4
11	♀ 1,2,3	♂ 1,2,3.....	2,000	11	10	1.1	1.0
12	y Cy/Pm; H/Sb.....		997	7	1	1.4	0.2
13	ClB; Cy/Pm; H/Sb.....		1,304	3	..	0.4	...
14	MI.....		1,169	..	..	...	...

* Except in no. 2, the Y is disregarded, since no influence was found.

females, not only is the pod-X chromosome absent, but, in addition, a Y chromosome which inhibits female penetrance in the absence of the sex-linked factor is present.

Nos. 3, 4, 8 show the effects of the isolated first, second, and third chromosomes, respectively. (The Y chromosome is disregarded, because in nos. 1, 5, 6, in which the pod Y alternates between females and males, no difference in penetrance was found.) Of these, the first chromosome has the smallest effect, next comes the third, and the highest effect is found for the second. Only the last shows the great sexual difference in penetrance which is typical for pod G. The effect of the combination of first and third (no. 10) is higher than the added single effect of these chromosomes. But the effect of the combination of first and second chromosomes, which ought to be higher than 2 acting separately, is actually even lower for the females and extremely low for the males. If we compare the males of nos. 5 and 6, which have the same constitution as the males of nos. 9 and 10, they come nearer to expectation. If we add all males of the same constitution, we get for chromosomes 1 = 0.6,

2 = 1.9, 3 = 1.0, 1 + 3 = 2.1, 2 + 3 = 1, 1 + 2 = 2.1. Altogether, the numbers do not give much insight into the quantitative collaboration of these factors. The greatest disappointment is no. 11, in which all pod chromosomes (except the fourth) have been reunited and the penetrance of the original line (more than 3 per cent) should be restored. Instead, a very low penetrance is found which cannot be explained, because certainly the first and second chromosomes could not have been changed by crossing over in the course of the experiment.

TABLE 76

ISOLATED CHROMOSOMES OF tet, SELECTED STOCK

(Numbers and Y in second column indicate the tet chromosomes present. $\underline{X}$ = foreign attached X chromosomes)

No.	Chromosomes		+	tet ♀	tet ♂	pen ♀	pen ♂	tet ♀ : 1 ♂
1	♀ $\underline{X}$	♂ 1Y alternates . . .	1,771	11	57	1.2	6.2	0.2
2	♀ $\underline{XY}$	♂ Y	1,241	3	5	0.5	0.8	0.6
3	♀ $\underline{XY}2$	♂ Y2	3,432	67	43	3.9	2.4	1.6
4	♀ $\underline{XY}3$	♂ Y3	1,221	3	12	0.5	1.9	0.3
5	♀ $\underline{X}2$	♂ 1,2Y alternates . .	626	3	6	1.0	1.9	0.5
6	♀ $\underline{X}3$	♂ 1,3Y alternates . .	1,553	15	50	1.9	6.2	0.3
7	♀ $\underline{X}2,3$	♂ 2,3Y alternates . .	1,506	14	12	1.8	1.5	1.2
8	♀ 1	♂ 1	2,627	35	61	2.6	4.5	0.6
9	♀ 1,2	♂ 1,2	1,145	68	42	10.9	6.7	1.6
10	♀ 1,3	♂ 1,3	3,022	41	103	2.8	6.5	0.4
11	♀ 1,2,3	♂ 1,2,3	546	41	69	12.5	21.0	0.6

b. *Tetraltera*

Table 76 presents the facts for a tetraltera line which was selected for high penetrance but could not be considered to be completely homozygous. Here the second-chromosome factor has the strongest action when by itself. This is seen in no. 3 in the females, although the value is below expectation because of the Y-chromosome action. The male value is here very low also, showing that, just as in podoptera, the second chromosome controls a higher female penetrance, and that the sex ratio in favor of males, which is typical for tet, is a product of the first-chromosome factor (no. 8). The third-chromosome factor has again a low effect (no. 4). In the collaboration of two factors we find for 2 and 3 (no. 7) a lower penetrance than we do for 2 separate factors. First and third chromosomes together have added their effects. In the combination 1, 2 we find a higher effect than the sum of both chromosomes operating singly and, as typical in the presence of 2, a higher female effect. But in no. 5, in which the males are also 1, 2, a very low penetrance appears. When all three chromosomes are brought together again after isolation, penetrance is increased beyond the added values, especially so in the males. We may compare these data for the males in the following diagram (fig. 35) which shows a roughly additive effect of 1 + 3, a diminished effect of 2 + 3, and an exponential effect of 1 + 2 + 3. The data for the females are, however, more irregular.

The most striking discrepancy in these results appears when they are compared with the outcrossing experiments. The isolation of the chromosomes was done with flies from the same selected line which had an average penetrance of 50–70 per cent. After ordinary outcrosses with this line, the high penetrance could easily be recovered by selection in a few generations (see above). Such experiments were repeated many times, e.g., when $y\ cv$ or y were put into the tet stock. Each time, the high penetrance was recovered. Why is it not recovered here when the stock is reconstituted again from individual chromo-

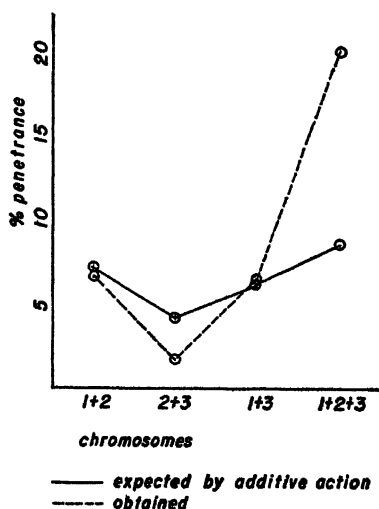


Fig. 35. Effect of different combinations of isolated chromosomes upon penetrance in tetraltera.

somes which were shown to contain the tet factors? Something must have happened during the outcrosses with dominant markers. Crossing over in the first and second chromosomes was excluded. The third, in which some crossing over may have occurred, is the least important one. The fourth chromosome had been shown not to contain a major tet factor. The cytoplasm is indeed foreign cytoplasm, but the numerous reciprocal crosses with tet never showed any marked influence of the cytoplasm. The assumption that by chance the second-chromosome factor had mutated to a lower allele in the male first used for this isolation might be an explanation, but it is not too satisfactory. There is available one test which might shed light on this difficulty. In table 79 below (a table concerned with testing possible cytoplasmic influence), tet males are produced by combining the isolated second and third tet chromosomes with all the chromosomes of the tet stock (nos. 1, 2, in the table). These males differ from those in the set just discussed by being heterozygous for the fourth chromosome of tet and, possibly, for exchanged sections of the third chromosome, which is not completely protected from crossing over. Actually, the penetrance is almost twice as great as in the example discussed above. This might mean that the fourth chromosome, which when alone does not

seem to contain a tet factor, actually has one, which, even in heterozygous condition, collaborates with the tet factors in the other chromosomes if all are present. A case of collaboration of a homozygous pod factor with another heterozygous one was found in pod-H (p. 218).

In table 77 a chromosome-isolation experiment performed with the tet-100 stock is tabulated. The autosomes here are the same as before, but the powerful first chromosome (with 2 tet factors) is different. Again, there are great discrepancies in the data. Thus, the males in no. 1 and no. 8 are genetically identical, yet the results are different. The absence of tet females in no. 8

TABLE 77
ISOLATION OF tet-100 CHROMOSOMES
(Explanation as in tables 75, 76)

No.	Chromosomes		+	tet ♀	tet ♂	pen ♀	pen ♂
1	♀ $\underline{X}$	♂ 1,Y alternates.....	667	7	9	2.0	2.6
2	♀ $\underline{XY}$	♂ Y.....	701	2	2	0.6	0.6
3	♀ $\underline{XY2}$	♂ Y2.....	1,119	11	19	1.9	3.3
4	♀ $\underline{XY3}$	♂ Y3.....	1,391	7	2	1.0	0.3
5	♀ $\underline{X2}$	♂ 1,2Y alternates.....	1,133	11	24	1.9	4.1
6	♀ $\underline{X3}$	♂ 1,3Y alternates.....	866	1	..	0.2	...
7	♀ $\underline{X2,3}$	♂ 2,3Y alternates.....	1,178	1	16	0.2	2.7
8	♀ 1	♂ 1.....	1,057	..	3	...	0.6
9	♀ 1,2	♂ 1,2.....	1,408	38	45	5.1	6.1
10	♀ 1,3	♂ 1,3.....	1,041	9	49	1.6	8.9
11	♀ 1,2,3	♂ 1,2,3.....	16	33	65	71.1	94.2

among more than 1,000 individuals, is unexplained. The same is true for no. 6, with hardly any tet flies, although the males of no. 10 of the same composition have the expected penetrance. One might at first think that an error had been made in the chromosome isolation. But this is excluded by the result of no. 11, in which the recombination of 1, 2, 3 out of isolated chromosomes returns penetrance to almost the original value. In fig. 36 the comparison of the results for males has been made in a parallel way to that of fig. 35. The exponential action is visible for the 1 + 3 combination, and 1 + 2 + 3 raises penetrance to 94.2 per cent. It is of no use to push the analysis much further. Unknown factors, it is clear, control the results. In table 59 the individual actions of tet factors for each chromosome were estimated on the basis of selection experiments. The tet action of both individual factors and that of two combined factors was much higher than that found in the present isolation experiment. The differences between these two experiments are: first, the presence of the dominant markers, and second, the absence of heterozygosity of tet factors, the homozygous isolated tet chromosomes being the only part of the tet genome in the present experiment. A part of the discrepancy may be attributed to these two features. Even so, some of it is still unexplained.

One of the possibilities which were tested was that a segregating fourth-chromosome factor may have a larger influence than had been assumed (see

the preceding discussion of this point). If this is true, it is possible that individuals of tet phenotype have such factors, and therefore, selection of tet phenotypes should increase penetrance. This was done for two generations, for the combination tet-100, 1, 2. The control is no. 9 in table 77. The result is tabulated in table 78. Actually, a small increase in penetrance was accom-

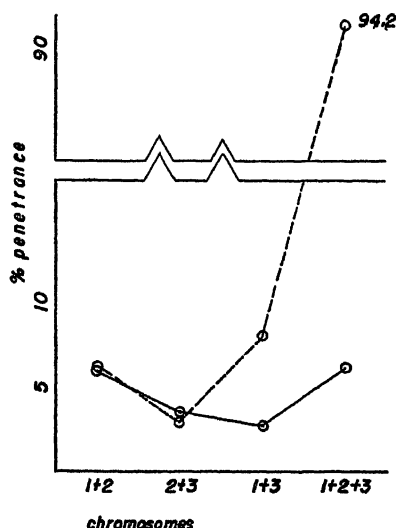


Fig. 36. Effect of different combinations of isolated chromosomes upon penetrance in tet-100.

TABLE 78
SELECTION OF tet PHENOTYPES IN tet-100 1, 2

	+	tet	Penetrance
P.....	1,408	83	5.5
F ₁	185	26	12.3
F ₂	389	69	15.1

plished, which is in favor of the explanation involving the fourth chromosome. It is regrettable that a direct test is prevented by the inviability of stocks with all chromosomes marked by dominants.

We mentioned above the possibility that the cytoplasm may have an influence upon the result. Therefore, a check was made by making identical combinations differing only in their cytoplasm, namely, reciprocal crosses between the tet stocks and the lines with isolated second and third chromosomes, the latter having foreign cytoplasm (from the y stock). Table 79 contains the results, which are negative. (It should be explained that the penetrance of the tet-100 males, which is identical here with that of ordinary tet, is a consequence of a return mutation which has occurred in the first chromosome of this stock, as already reported, and has made the X chromosome again identical with that of the original tet stock.)

TABLE 79
TESTS FOR CYTOPLASMIC INFLUENCE
(1^t, etc., means first, etc., chromosome derived from tet. In nos. 3, 4, all t = t-100)

Cross	Cyto- plasm	Chromosomes		♀		♂	
		♀	♂	+	tet	+	pen.
1. tet stock × tet-2, -3.....	tet	1/1 ^t 2 ^t /2 ^t 3 ^t /3 ^t 4 ^t /4 ^t	1 ^t 2 ^t /2 ^t 3 ^t /3 ^t 4 ^t /4 ^t	746	14	393	42.6
2. Reciprocal	Σ	1/1 2 ^t /2 ^t 3 ^t /3 ^t 4 ^t /4 ^t	1 ^t 2 ^t /2 ^t 3 ^t /3 ^t 4 ^t /4 ^t	82	1	63	40.0
3. tet-100 stock × tet-100 2, 3.....	tet	1/1 ¹⁰⁰ 2 ^t /2 ^t 3 ^t /3 ^t 4 ^t /4 ^t	1 ¹⁰⁰ 2 ^t /2 ^t 3 ^t /3 ^t 4 ^t /4 ^t	346	13	199	44.4
4. Reciprocal.....	Σ	1/1 2 ^t /2 ^t 3 ^t /3 ^t 4 ^t /4 ^t	1 ¹⁰⁰ 2 ^t /2 ^t 3 ^t /3 ^t 4 ^t /4 ^t	245	7	213	43.7

c. Podoptera H

A different method of isolating the replacement lines in pod-H had to be exercised for two reasons: (1) The balanced semilethal, sterile condition of the third chromosomes (see footnote p. 72) made it virtually impossible to obtain any homozygous pod-H third-chromosome lines. Therefore, crosses had to be made in such a way as to retain both the third chromosomes and yet have the X and second homozygous if possible. (2) The data had consistently shown that the direction in which the crosses were made affected the expression of podoptera, so a third series of lines was isolated in which the cytoplasm was originally derived from a pod-H female. The following method was devised to set up the three groups:

(1) $\underline{y}$ Cy/Pm; H/Sb ♀ was mated with a pod-H ♂ (from a line inbred 23 generations). This gave females with foreign attached X chromosomes and Y^p and X^pY males (X^p meaning X from pod.)

(2) ClB/X; Cy/Pm; H/Sb ♀ mated to the same ♂ gave ClB/ X^p females and XY^p males. By crosses between appropriate females and males from these two lines during subsequent generations, a number of lines were isolated with the following combinations of the first chromosomes: $\underline{y}Y$, $\underline{y}Y^p$, XX , X^pX^p and XY , X^pY , XY^p and X^pY^p , as well as the various combinations of the second and third chromosomes.

(3) A pod-II ♀ was mated with an XY Cy/Pm H/Sb ♂, giving X^pX females and XY^p males. The ♂ was backcrossed to a pod-H ♀, thus eliminating the nonpod X chromosome; heterozygotes were selected and mated either to brothers or to X^pY^p males derived from the other lines, to give X^pX^p females and X^pY or X^pY^p males combined with the appropriate second and third chromosomes. Because of the lack of markers in the foreign X chromosomes, heterozygotes could not be distinguished; consequently no pod lines with nonpod X chromosomes were isolated.

In the tables the same designation is used as above in the pod-G replacement lines, e.g., only the pod chromosomes are designated, X or 1 for the pod-X chromosome, Y for the pod-Y chromosome, 2 for the homozygous pod second chromosomes, and 3 for the pod third chromosomes. The attached X females marked with yellow are designated $\underline{y}$ or $\underline{X}$ if they have a pod-Y in addition, as $\underline{y}Y$, or $\underline{X}Y$; if a foreign Y only, as $\underline{y}$ or $\underline{X}$ (table 80). Likewise, the isolated lines were bred by single-pair matings for 3 or 4 generations, 10 matings per generation. However, it was impossible to obtain all the 40 possible lines, because flies with certain of the combinations of chromosomes were so nearly sterile that the lines could not be isolated or gave so few flies that the data could not be used for comparative purposes. Therefore, the following analysis is based only upon the lines that had 500 or more flies, with the exception of the lines in which the incidence of podoptera was high enough to make the data significant. For the sake of convenience, the lines that are descendants in the maternal line of $\underline{y}$; Cy/Pm; H/Sb females are designated $\underline{X}$; those from ClB; Cy/Pm; H/Sb females, ClB (Cytopl. ClB) and those from the pod-H ♀, pod (Cytopl. pod).

(1) *Control lines*.—Two of the lines (nos. 34, 35) had all the pod chromosomes replaced by marker chromosomes except the fourth, which may have been heterozygous or homozygous pod or nonpod. Comparison of these two lines

TABLE 80
ISOLATION OF CHROMOSOMES OF pod-H
(Explanation as in foregoing tables)

No.	Chromosomes		Total	pod ♀	pod ♂	pen ♀	pen ♂
1	♀ $\bar{X}$	♂ 1.....	1,204	3	3	0.5	0.5
2	♀ $\bar{X}Y$	♂ Y....	1,121	1	2	0.2	0.4
3	♀ $\bar{X}2$	♂ 1,2.....	205	3	2	3.0	2.0
4	♀ $\bar{X}3$	♂ 1,3.....	1,064	1	5	0.2	0.9
5	♀ $\bar{X}3$	♂ 3.....	1,202	2	2	0.4	0.3
6	♀ $\bar{X}Y2$	♂ Y2.....	23	..	..	..	..
7	♀ $\bar{X}Y3$	♂ Y3.....	696	4	5	1.2	1.4
8	♀ $\bar{X}Y2,3$	♂ Y2,3.....	1,490	2	4	0.3	0.5
9	♀ $\bar{X}Y$	♂ Y1.....	494	9	2	3.6	0.8
10	♀ $\bar{X}Y2$	♂ Y1,2.....	751	21	31	5.6	8.2
11	♀ $\bar{X}Y3$	♂ Y1,3.....	1,070	..	2	..	0.4
12	♀ $\bar{X}Y2,3$	♂ Y1,2,3.....	1,722	8	8	0.9	0.9
13	♀ 2	♂ 2.....	1,037	75	40	14.5	7.7
14	♀ 3	♂ 3.....	1,161	1	..	0.2	..
15	♀ 2,3	♂ 2,3.....	1,944	15	8	1.6	0.8
16	♀ 2	♂ Y,2.....	1,025	53	35	10.3	6.9
17	♀ 3	♂ Y,3.....	1,294	..	2	..	0.3
18	♀ 2,3	♂ Y2,3.....	2,117	5	8	0.5	0.8
19	♀ none	♂ Y.....	1,109	2	2	0.3	0.3
20	♀ 1	♂ 1 (Cytopl. ClB).....	1,121	5	3	0.9	0.5
21	♀ 1,2	♂ 1,2 (Cytopl. ClB).....	953	31	16	6.5	3.4
22	♀ 1	♂ 1 (Cytopl. pod).....	760	8	4	2.1	1.1
23	♀ 1,2	♂ 1,2 (Cytopl. pod).....	881	19	11	4.3	2.5
24	♀ 1,3	♂ 1,3 (Cytopl. pod).....	1,031	..	..	..	..
25	♀ 1,2,3	♂ 1,2,3 (Cytopl. pod)...	1,358	35	27	5.2	4.0
26	♀ 1	♂ Y1 (Cytopl. ClB).....	1,236	1	5	0.1	0.8
27	♀ 1,2	♂ Y1,2 (Cytopl. ClB).....	1,078	80	66	14.9	12.2
28	♀ 1,3	♂ Y1,3 (Cytopl. ClB).....	1,257	..	3	..	0.5
29	♀ 1,2,3	♂ Y1,2,3 (Cytopl. ClB)....	2,939	52	30	3.5	2.0
30	♀ 1	♂ Y1 (Cytopl. pod).....	1,512	3	2	0.4	0.3
31	♀ 1,2	♂ Y1,2 (Cytopl. pod).....	1,928	93	105	9.7	10.9
32	♀ 1,3	♂ Y1,3 (Cytopl. pod).....	1,540	4	..	0.5	..
33	♀ 1,2,3	♂ Y1,2,3 (Cytopl. pod)....	2,626	25	28	1.9	2.2
34	♀ $\bar{X}$	♂ none (Cytopl. $\bar{X}$).....	1,074	2	2	0.4	0.3
35	♀ none	♂ none (Cytopl. ClB).....	1,168	2	..	0.3	..

with the control lines of the marker stock (table 81) shows that there is probably no significant difference in incidence of podoptera between controls and pod-H lines with similar genotypes (although the number may be too small to make a statistical test of any value). From these data it seems obvious that the primary pod-H factor is not in the fourth chromosome, because if it were, the pod-H line would have a higher incidence of podoptera than the control line.

Second, the marker stocks have a low incidence of podoptera ($\bar{y}$, 0.79; ClB, 0.22) which is not changed significantly by combination with pod and subsequent extraction; therefore, pod factors must be present in the marked chromosomes. Third, the incidence of podoptera is greater in the $\bar{X}$ marked line than in the ClB marked line. Since $\bar{y}$; Cy/Pm; H/Sb was derived from the ClB; Cy/Pm; H/Sb stock except for the $\bar{y}$ chromosomes the increased incidence of podoptera is probably due to the attached X chromosomes or to the

TABLE 81

COMPARISON BETWEEN MARKER STOCK CONTROL LINES AND pod-H LINES WHICH HAVE THE X, Y, SECOND AND THIRD CHROMOSOMES REPLACED

	Total flies	Podoptera			χ^2
		♀	♂	Percentage	
Control: $\bar{X}$ Cy/Pm; H/Sb;.....	1,005	7	1	0.79	1.02
$\bar{X}$ line: $\bar{X}$ Cy/Pm; H/Sb; 4?.....	1,074	2	2	0.37	
Control: ClB; Cy/Pm; H/Sb.....	1,367	3	..	0.22	
ClB line: Cy/Pm; H/Sb 4?.....	1,168	2	..	0.17	

TABLE 82

INCIDENCE OF PODOPTERA H WITH COMBINATIONS OF THE SECOND AND THIRD CHROMOSOMES—ClB AND Pod LINES

	ClB lines			Pod lines			Percentage of pod
	Total flies	pod	Percentage	Total flies	pod	Percentage	ClB+pod
2 and 3 replaced.....	4,634	20	0.43	2,272	17	0.75	0.54
2 replaced, 3 pod.....	3,712	6	0.16	2,517	4	0.16	0.16
2 pod, 3 replaced.....	4,093	396	9.67	2,809	228	8.12	9.04
2 pod, 3 pod.....	7,000	118	1.69	3,984	115	2.89	2.13

combinations of the attached X with Y. From these data the following generalizations seem warranted: (1) the fourth chromosome may be neglected without decreasing the significance of the data and subsequent conclusions. (2) The incidence of podoptera in the control $\bar{X}$ lines is so much higher that the experimental $\bar{X}$ lines must be considered separately from the ClB and pod lines.

(2) *The second-chromosome podoptera factor.* — The data of table 80 are arranged in table 82 to compare the effect of the second and third chromosomes on the expression of podoptera. From the combined data for the ClB and Pod groups, it becomes evident that the primary factor is in the second chromosome (replaced 2 and replaced 3 gave 0.54 per cent; replaced 2, pod-3, 0.16 per cent; pod-2, replaced 3, 9.04 per cent; pod-2, pod-3, 2.13 per cent) although a low incidence of podoptera flies may still occur when the second pod chromosome is replaced.

The high incidence of podoptera when the second chromosome is combined with Hairless and Stubble is as marked as it was in the first localization experiment with dominant markers. The data are deceptive and are difficult to evaluate because the interpretation may be either enhancing effect or pod factors in the II/Sb chromosomes, or inhibiting factors in the pod third chromosomes. However, the results from the previous experiment (p. 186) indicated that Hairless and Stubble individually had podoptera-enhancing effects; therefore, the increase in penetrance from 2.13 per cent in the lines with pod-2, pod-3 to 9.04 in the pod lines with replaced 2, 3 is probably due to the enhancing effects of the Hairless and Stubble chromosomes. This hypothesis is further substantiated by the fact that the H/Sb combination

TABLE 83
EFFECT OF X AND SECOND CHROMOSOMES UPON THE EXPRESSION OF PODOPTERA H

Group	Chromosomes	CIB lines			Pod lines		
		Total flies	pod	Percentage	Total flies	pod	Percentage
I	X and 2 replaced	4,732	9	0.19			
II	X pod, 2 replaced	3,614	17	0.47	1,843	21	0.43
III	X replaced, 2 pod	6,123	239	3.90			
IV	X pod; 2 pod	4,970	275	5.53	6,793	343	5.05

does not have a generalized effect, that is, it does not increase the penetrance of all 6 classes of podoptera but acts primarily in increasing the incidence of class II (wing blade modifications).

(3) *The X-chromosome podoptera factor*.—The data given in table 80 are arranged in table 83 to compare the effect of the X and second chromosomes upon the penetrance of podoptera. The most striking fact brought out by this arrangement is that the penetrance increases with the addition of pod chromosomes, e.g., in the CIB series, replaced X and replaced 2 has 0.19 per cent podoptera; pod-X, replaced 2, 0.47 per cent; replaced X, pod-2, 3.90 per cent; and pod-X, pod-2, 5.53 per cent. The pod lines show no deviations. These data show that the X chromosome has an independent pod effect (χ^2 of group I to II for CIB is 5.30 per cent) and acts also as an enhancer of the primary pod factor in the second chromosome (or collaborates with it in producing the pod effect).

This evaluation, however, is based on the assumption that the groups are strictly comparable without taking into consideration the discrepancies due to variations in numbers of flies in the separate lines, or the deviations that may be caused by the large number of podoptera in lines with pod-2, replaced 3. In order to minimize as many of the variables as possible, we have rearranged the data from groups III and IV of table 83 in table 84 to show the contrast between the effect of the pod-X and the replaced X in lines having the pod second and third chromosomes, and have rearranged them in table 85 to make a similar comparison of lines having the pod second chromosome but

the third replaced. If both the second and third chromosomes are from pod, the difference between the pod-X and nonpod-X lines is highly significant ($\chi^2 = 46.41$), but if the third chromosome is replaced by H/Sb, the difference is not significant. If the percentage values are considered in this case, the lines with pod-X actually have a lower incidence of podoptera (replaced X, 9.84 per cent; pod-X, 8.49 per cent). These data suggest that pod factors are

TABLE 84
EFFECT OF pod-X AND nonpod-X ON THE EXPRESSION OF PODOPTERA H IN LINES
WITH pod-2 and pod-3

	CIB		Pod		Totals		Percentage
	Total flies	pod	Total flies	pod	Total flies	pod	
X replaced	4,061	36			4,061	36	0.89
X pod	2,939	82	3,984	115	6,923	197	2.85
χ^2	36.62 ⁺				46.41 ^{**}		

** Following the method used by Snedecor, we use a double asterisk to denote the probability beyond the 1 per cent level, a single asterisk for the probability between the 5 per cent and 1 per cent levels.

TABLE 85
EFFECT OF pod-X AND nonpod-X ON THE EXPRESSION OF PODOPTERA IN LINES
WITH pod-2, AND 3 REPLACED

	CIB		Pod		Totals		
	Total flies	pod	Total flies	pod	Total flies	pod	Percentage
X replaced	2,062	203			2,062	203	9.84
X pod	2,031	193	2,809	228	4,840	421	8.49
χ^2	0.26				1.80		

present in both pod- and nonpod-X chromosomes, but they enhance only the pod factor in the autosomes of their own line (they collaborate with them). An alternative explanation may be that the nonpod-X has no podoptera effects but the pod-X inhibits the expression of podoptera in nonpod chromosomes.

By further analysis of the data in table 80, it was possible to show (table 86) that (in the CIB lines, with pod-2 or pod-2, pod-3) when the pod-X alone (without pod-Y) is contrasted with the nonpod-X (either with or without the pod-Y), the higher penetrance is found in the nonpod-X lines, and that the difference is statistically significant. But if the pod-X genotype also includes the pod-Y, the penetrance is significantly higher in the pod-X lines. It therefore appears as if the Y chromosome is an important factor in the expressivity of podoptera, at least in the lines which had pod-2, replaced 3. Unfortunately, the critical data are not available for comparison of pod-2, pod-3 to pod-X,

TABLE 86

COMPARISON OF EFFECTS OF pod-X AND nonpod-X IN THE EXPRESSION OF PODOPTERA H IN THE CIB LINES

Compared	nonpod-X			pod-X			χ^2	Significance
	Total	pod	Percentage	Total	pod	Percentage		
2,3 to XY2,3.....	1,944	23	1.18	2,939	82	2.79	14.30	Higher in pod-X
Y2,3 to XY2,3.....	2,117	13	0.61	2,939	82	2.79	31.27	Higher in pod-X
2 to X2.....	1,037	115	11.08	953	47	4.93	23.40	Higher in nonpod-X
2 to XY2.....	1,037	115	11.08	1,078	146	13.54	2.55	Not significant
Y, 2 to X2.....	1,025	88	8.58	953	47	4.93	9.40	Higher in nonpod-X
Y, 2 to XY2.....	1,025	88	8.58	1,078	146	13.54	12.16	Higher in pod-X
Y3 to XY3	1,294	2	0.15	1,257	3	0.24		
3 to XY3.....	1,161	1	0.08	1,257	3	0.24		
nonpod to X.....	1,168	2	0.17	1,121	8	0.71	2.71	Not significant
nonpod to XY.....	1,168	2	0.17	1,236	6	0.48		Not significant
Y to XY.....	1,109	4	0.36	1,236	6	0.48	0.02	Not significant
Y to X.....	1,109	4	0.36	1,121	8	0.71	0.76	Not significant

pod-2, pod-3. Nor can the lines with replaced 2, pod-3 be compared, because of the low incidence of podoptera. However, the data do suggest that the combinations pod-X, pod-Y do enhance the expression of the third-chromosome pod factor. The ClB lines with nonpod-X, 2 and 3 replaced, compared to lines with pod-X and 2, 3 replaced, likewise had no statistically significant difference, although the lines with pod-X consistently have a higher percentage incidence of podoptera flies. Therefore, it may be postulated that the primary effect of the pod-X is that of an enhancer of the expression of the primary factor in the second chromosome. It probably enhances the expression of the third-chromosome factor as well. Finally, the pod-X chromosome has independent pod effects when the second chromosome is replaced.

TABLE 87

EFFECT OF THE pod-X AND pod-3 CHROMOSOMES UPON THE EXPRESSION OF PODOPTERA

	ClB			Pod		
	Total	pod	Percentage	Total	pod	Percentage
X and 3 replaced.....	4,339	209	4.82		...	
X pod; 3 replaced..	4,388	207	4.72	5,081	245	4.82
X replaced; 3 pod..	6,516	39	0.60		...	
X pod; 3 pod.....	4,196	85	2.03	6,555	119	1.82

(4) *The third-chromosome podoptera factor.*—The results obtained in the analysis of the second-chromosome pod factor showed that replacement of the third chromosome by Hairless/Stubble (nonpod third chromosomes) resulted in increased incidence of podoptera (table 82). On the other hand, when the second chromosome was replaced, the incidence of podoptera was still 0.16 per cent. This low podoptera effect could be explained in three ways: (1) expression of the podoptera factors in the marker chromosomes, (2) the pod-X chromosome effect, or (3) a very low pod effect of the pod third chromosome. In the comparison of replacement of pod-X and pod-2 (table 83) when both the X and second pod chromosomes were replaced the incidence of pod was 0.19 per cent, but when the X was pod and the second was replaced, the incidence was 0.47 per cent. These data could be explained on the basis of (1) marker-chromosome pod effects in the first case and pod-X effects in the second case, or (2) a very low pod effect in the third chromosome which is enhanced or supplemented by the pod-X effect. To test the hypothesis that the third chromosome had a podoptera effect, the data of table 80 are rearranged in table 87 to compare the podoptera effects of the pod-X and pod-3 chromosomes. As would be expected, from the previous considerations, replacement of the third chromosome results in a relatively high incidence of podoptera owing to the pod-enhancing effects of the Hairless and Stubble chromosomes. When the third is pod and the X is replaced, the incidence is 0.60 per cent (ClB lines alone), but when the X and third are both pod the incidence is 1.90 per cent (2.03 per cent in ClB, and 1.82 per cent in pod lines).

Any other comparisons are not feasible, because the data are too meager to make a statistical analysis of any value; however, they do suggest that there is a third-chromosome factor, but its expression is dependent upon the presence of either or both the first and second podoptera chromosomes.

A better proof of the existence of a third-chromosome pod factor was obtained, however, in a different way. In the course of the inbreeding experiments, it was noted that some of the flies were simultaneously podoptera and Minute-like; likewise that nonpodoptera Minute-like flies appeared consistently but always in very low numbers. If the Minute-like flies could be bred (they were always very weak), they gave normal offspring in the first genera-

TABLE 88

INCIDENCE OF MINUTE-LIKE AND MINUTE-LIKE PODOPTERA FLIES IN THE ClB AND Pod REPLACEMENT LINES OF pod-H

Line	pod chromosomes	Total flies	Total pod		M pod		M nonpod	
ClB	3	1,161	1	..	..	..	..	1
ClB	Y3	1,294	..	2	..	1	2	2
ClB	Y2,3..	2,117	5	8	..	..	..	1
ClB	XY2,3	2,937	52	30	..	..	2	2
Pod	X3...	1,031	..	..	..	..	2	4
Pod	X2,3...	1,358	35	27	..	..	1	1
Pod	XY3....	1,540	4	..	1	..	..	4
Pod	XY2,3..	2,626	25	28	1	..	..	1

tion, but Minute-like flies appeared in later generations. Although this shows that no real Minute was involved, the incidence of Minute-like and Minute-like podoptera flies was checked. The data are summarized in table 88. The Minute-like effect was found only when a pod-3 chromosome was present. Therefore, the Minute-like factor must be in one of the pod third chromosomes and is a dominant with low penetrance. But as Minute-like recessives are known to exist (Mohr, 1925) it might also be postulated from these data that the Minute-like factor is a recessive of high penetrance and is expressed only on the rare occasions when the Minute-like locus in the third chromosome becomes homozygous as the result of crossing over. A decision between the two hypotheses cannot be made from the data of the replacement experiment. If the data from the localization experiments, summarized in table 89, are considered, the second hypothesis would be applicable only if the further assumption is made that the marked third chromosomes contain Minute alleles recessive to the Minute-like factor in the pod-3 chromosome, or that the dominants act as enhancers of dominance of Minute. But if Minute recessives are present in the Hairless and Stubble chromosomes which are alleles of an assumed Minute factor in the pod chromosomes, they cannot be alleles of each other, because Minute podoptera flies are not found among pod flies with H/Sb. Whatever the method of inheritance of the Minute-like factor, it is plain from these data that a very low penetrance Minute-like

factor is present in the third chromosome which under certain conditions will give a pod effect.

In the preceding discussion no account was given of the relations of this factor to the pod factors in the first and second chromosomes. By further consideration of the data in tables 88 and 89 it becomes apparent that the Minute-like effect may be expressed in the absence of the pod-X and pod-2 chromosomes, but that the Minute-like and pod flies are in the lines that have a pod-Y in the genotype. The Y chromosome must play a role in the expression of the Minute-like pod phenotype. Details of the role played by the Y in the expression of podoptera will be presented below.

TABLE 89
EXPRESSION OF MINUTE-LIKE pod PHENOTYPES IN FLIES HAVING VARIOUS
COMBINATIONS OF pod AND DOMINANT MARKER CHROMOSOMES
(Data extracted from localization experiment)

Phenotypes	Total flies	Total pod non M		Total M pod		Y Chromosome
		♀	♂	♀	♂	
+ (all pod)	2,302	15	15	1	..	Y
Cy Pm.	805	1	1	1	1	Y ^p
Cy	1,330	4	.	1	..	Y ^p
Pm.	1,395	7	5	2	..	Y ^p
Cy H.	905	2	2	1	..	Y ^p
Cy Sb.	819	3	2	..	1	Y ^p
Sb.	1,058	15	9	..	4	Y or Y ^p
H Sb.	935	39	18	..	..	Y or Y ^p

(5) *The effect of the pod-Y chromosome upon the penetrance of podoptera H.*—In a later chapter the general effects of the Y chromosome upon podoptera penetrance will be studied. Here a specific effect of pod-Y as opposed to a foreign Y will be reported.

Certain discrepancies had been found in the analysis of the pod-X and pod-3 chromosomes, and their influence upon penetrance of podoptera, which could not be explained on the basis of the X, 2 and 3 chromosomes as being the only contributing factors in the production of the podoptera effect. In testing the effect of the X chromosome in lines with pod-2 but replaced 3 (table 86) it was established that the pod-X (without the pod-Y) lines had a lower incidence of podoptera than the nonpod-X lines, but if the lines had both pod-X and pod-Y the incidence was higher than in the nonpod-X lines. In the case of the third chromosome (tables 88, 89), the Minute-like phenotype could appear with or without the presence of the pod-X, pod-Y, and pod-2 chromosomes, but the Minute-like flies were only present in the lines which contained the pod-Y chromosomes. An explanation had been based upon the yet unproved assumption that the Y plays an important role in the expression of podoptera. This assumption was tested by applying a similar analysis to the same data as in the earlier experiments, but in this case contrasting the effects of the presence and absence of the pod Y on the expression of podoptera.

In order to ascertain whether the difference (which was highly significant) between the pod-X and nonpod-X in the lines with pod-2 and pod-3 was due entirely to the X chromosome, certain data of table 80 were rearranged in table 90 to contrast the effect of pod-Y to nonpod-Y. The most striking facts brought out in this comparison are: in the ClB group the pod-Y lines have an incidence of 1.88 per cent and the lines with replaced Y, 1.18 per cent, the difference between them being statistically significant; whereas in the Pod

TABLE 90
EFFECT OF pod-Y AND nonpod-Y ON THE EXPRESSION OF PODOPTERA II IN LINES
WITH pod-2 AND pod-3

	ClB			Pod		
	Total flies	pod	Percentage	Total flies	pod	Percentage
Y replaced.....	1,944	23	1.18	1,358	62	4.57
Y pod.....	5,056	95	1.88	2,626	53	2.03
χ^2	4.30*			20.32**		

TABLE 91
EFFECT OF COMBINATIONS OF pod-X AND pod-Y ON THE INCIDENCE OF PODOPTERA II IN
LINES WITH pod-2 AND pod-3

Line	Compared	Total flies	pod	Percentage	Total flies	pod	Percentage	χ^2	Significance
ClB	2,3 to Y2,3.....	1,944	23	1.18	2,117	13	0.61	3.03	Not significant
ClB	2,3 to XY2,3.....	1,944	23	1.18	2,939	82	2.79	14.30	Higher in XY
ClB	Y2,3 to XY2,3...	2,117	13	0.61	2,939	82	2.79	31.27	Higher in XY
Pod	X2,3 to XY2,3...	1,358	62	4.57	2,626	53	2.03	20.32	Higher in X

group the incidence of podoptera in pod-Y lines (2.03 per cent) is lower than in the nonpod-Y lines (4.57 per cent) and the difference is highly significant. Possible causes for these new discrepancies may be determined by arranging the same data in table 91 in such a way as to compare as far as possible the influence of the X and Y in all combinations (pod-Y, pod-X and pod-Y, pod-X and nonpod-X and Y). The results (summarized in the last column) show that there is no significant difference between the nonpod-Y lines and the pod-Y lines; the lines which had pod-X pod-Y have a higher incidence of podoptera than those without pod-X; and the line with pod X has a higher incidence than that with pod-X, pod-Y. The effect of pod-X (without the pod-Y) compared to nonpod-X could not be tested in these lines. In summary, it seems that the X and Y are both important in the expression of podoptera. The exact relationship is more clearly understood if comparisons of all lines are made (table 92).

Table 93 summarizing all available data is designed to contrast two lines at a time by placing the four combinations of X and Y in both the vertical

TABLE 92

EFFECT OF COMBINATIONS OF pod-X AND pod-Y COMBINED WITH pod-2 AND 3 OR pod-2, 3 REPLACED UPON THE EXPRESSION OF
PODOPTERA H IN FEMALES AND MALES

Group	Compared	♀						Summary
		Total	pod	Percentage	Total	pod	Percentage	
I	2,3 to Y2,3.....	965	15	1.55	1,143	5	0.44	Higher in nonpod
II	2,3 to XY2,3.....	965	15	1.55	1,609	52	3.23	Higher in XY
III	Y2,3 to XY2,3.....	1,143	5	0.44	1,609	52	3.23	Higher in XY
IV	pod X2,3 to XY2,3.....	653	35	5.36	1,328	25	1.88	Higher in X
V	2 to 2XY.....	600	75	12.50	512	80	15.63	Not significant
VI	2 to 2X.....	600	75	12.50	504	31	5.15	Higher in nonpod-X
VII	2Y to 2XY.....	561	53	9.63	512	80	15.63	Higher in XY
VIII	2 to 2Y.....	600	75	12.50	561	53	9.63	Not significant
IX	2X to 2XY.....	504	31	6.15	512	80	15.63	Higher in XY
X	pod 2X to 2XY.....	429	19	4.43	873	93	10.65	Higher in XY
XI	2X to 2Y.....	504	31	6.15	561	53	9.63	Not significant

Group	Compared	♂						Summary
		Total	pod	Percentage	Total	pod	Percentage	
I	2,3 to Y2,3.....	979	8	0.82	974	8	0.82	Not significant
II	2,3 to XY2,3.....	979	8	0.82	1,330	30	2.26	Higher in XY
III	Y2,3 to XY2,3.....	974	8	0.82	1,330	30	2.26	Higher in XY
IV	pod X2,3 to XY2,3.....	705	27	3.83	1,298	28	2.16	Higher in X
V	2 to 2XY.....	437	40	9.15	566	66	11.66	Not significant
VI	2 to 2X.....	437	40	9.15	449	16	3.56	Higher in nonpod-X
VII	2Y to 2XY.....	464	35	7.54	566	66	11.66	Higher in XY
VIII	2 to 2Y.....	437	40	9.15	494	35	7.54	Not significant
IX	2X to 2XY.....	449	16	3.56	566	66	11.66	Higher in XY
X	pod 2X to 2XY.....	425	11	2.63	1,055	105	9.95	Higher in XY
XI	2X to 2Y.....	449	16	3.56	1,464	35	7.54	Higher in Y

TABLE 93

SUMMARY OF EFFECT OF pod AND nonpod CHROMOSOMES UPON THE EXPRESSION OF PODOPTERA H IN CIB AND Pod LINES

	Auto- somes	Cyto- plasm	X pod, Y pod			X repl, Y pod			X pod, Y repl			X repl, Y repl		
			Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂
X repl Y repl	2	CIB	XY = repl	XY = repl	XY = repl	Y = repl	Y = repl	Y = repl	X < repl	X < repl	X < repl	per cent 11 08	per cent 12 50	per cent 9 15
	2,3	CIB	XY > repl	XY > repl	XY > repl	Y = repl	Y > repl	Y = repl	X	X	X	1 18	1 55	0 82
	repl	CIB	—	—	—	—	—	—	X = repl	—	—	0 17	0 34	
	3	CIB	—	—	—	—	—	—	X	X	X	0 08	0 17	
X pod Y repl	2	CIB	XY > X	XY > X	XY > X	Y > X	Y = X	Y > X	per cent 4 93	per cent 6 15	per cent 3 56			
	2,3	Pod	XY > X	XY > X	XY > X	X	X	X	3 41	4 43	2 43			
	repl	Pod	XY < X	XY < X	XY < X	X	X	X	4 57	5 36	3 83			
	3	CIB	XY = X	—	—	Y = X	—	—	0 71	0 92	0 51			
X repl Y pod	2	CIB	XY < X	XY < X	—	X	X	X	1 53	1 88	1 19			
	2,3	Pod	—	—	—	X	X	X						
	repl	CIB	XY > Y	XY > Y	XY > Y	per cent 8 58	per cent 9 63	per cent 7 54						
	3	CIB	XY > Y	XY > Y	XY > Y	0 61	0 44	0 82						
X pod Y pod	2	CIB	XY = Y	—	—	0 36	0 35	0 37						
	2,3	Pod	—	—	—	0 15		0 31						
	repl	CIB	per cent 13 54	per cent 15 63	per cent 11 66									
	3	Pod	10 24	10 65	9 95									
X pod Y pod	2	CIB	2 79	3 23	2 26									
	2,3	Pod	2 03	1 88	2 16									
	repl	CIB	0 45	0 16	0 82									
	3	Pod	0 33	0 39	0 27									
X pod Y pod	2	CIB	0 24											
	3	Pod	0 26	0 50	0 46									

Legend
= no significant difference
> greater than
< less than
repl replaced
— numbers less than 10.
x no data.

Legend
 = no significant difference
 > greater than
 < less than
 repl replaced
 — numbers less than 10.
 x no data.

and the horizontal columns. The actual percentage penetrance of podoptera for each line is given in the last column of each group (contrasting identical pairs, e.g., pod-X pod-Y to pod-X pod-Y). In the columns contrasting unlike pairs the results of the Chi-square test are given in tabular form (e.g., XY = repl. means that there was not a significant difference between pod-XY and replaced XY). From the results presented in this table it is possible to make the following generalizations concerning the effect of the X, Y, 2 and 3 pod and nonpod chromosomes upon the expression of podoptera:

(1) If both the second and third chromosomes are pod, the relative incidence of podoptera is highest of all if the X but not the Y is pod; it is intermediate if both X and Y are pod, or if both X and Y are replaced; and lowest if the Y but not the X is pod.

(2) The X chromosome has a greater pod effect than the Y if the second and third are pod, but the Y has more effect if the third is replaced by H/Sb.

(3) The nonpod-X and -Y have "specific" enhancers for the pod factors in the nonpod third chromosomes, as do the pod-X and pod-Y for the pod third-chromosome factor(s). This fact explains in part why there is a high incidence of podoptera if both the X and Y are pod or if both are nonpod, and only an intermediate incidence if one or the other is replaced.

(4) The ClB lines and the pod lines give essentially the same results; therefore, cytoplasmic factors are not present in the pod-H strain.

(5) Although the incidence of pod females is usually higher than that of pod males, no specific genetic factor seems to be responsible.

(6) *Effect of the Y chromosome in the female on penetrance in pod-H.*—An experiment, adding an extra Y chromosome similar to that for pod-G (see below) has thus far not been feasible, because of the very low penetrance of pod-H when combined with the proper markers, as well as the complications introduced by the maternal effect (see page 267). But some information concerning the effect of a Y in the female was obtained by making replacement lines with a stock containing attached X chromosomes as well as the dominant markers in the autosomes (y; Cy/Pm, H/Sb). These lines (designated X) differ from the ClB and pod replacement lines in that the females always have the foreign attached X chromosomes and, in addition, have a Y chromosome which may be either from pod-H or the marker stock. The males have either or both pod-X and pod-Y, depending again upon the type of replacement.

The reduction of penetrance of podoptera is evident in the X lines even in the unanalyzed data, and if these lines are compared to similar ClB and pod lines striking differences are observable. In table 94 the data of the X lines are arranged in the same way as for the ClB and pod lines (table 82) to compare the incidence of podoptera with the various combinations of the second and third chromosomes. If the pod-2 is present the incidence is lower in the X lines than in the ClB and pod lines and the difference is significant. But the incidence is higher in the X lines in which the second chromosome has been replaced than in the ClB and pod lines. In the X lines, alone, there is no significant difference between those in which pod-2 and pod-3 are replaced and those having pod-2 and pod-3. Finally, the two lines, repl.-2, repl.-3 and repl.-2,

pod-3, show no significant difference in incidence from the control lines (table 81). Although the inference from a consideration of these facts is that the principal factor is not in the second chromosome, further analysis showed that the discrepancies were due, in part, to the lack of critical lines, and in part to the relatively high incidence of podoptera phenotypes in the y; Cy/Pm, H/Sb stock.

The first comparison was made in order to determine whether the difference between the X lines and the ClB lines was due to the introduction of a non-pod-Y chromosome into the female genotype. A complete analysis could not be made, because of low incidence of podoptera in certain lines, and because

TABLE 91
EFFECT OF THE SECOND AND THIRD CHROMOSOMES UPON THE EXPRESSION OF
PODOPTERA IN THE X LINES

Line	X lines			ClB and Pod lines
	Total flies	pod	Percentage	Percentage of pod
2 and 3 replaced	3,893	21	0.62	0.54
2 replaced; 3 pod	4,122	21	0.51	0.16
2 pod, 3 replaced	987	57	5.81	9.04
2 pod; 3 pod	3,212	22	0.68	2.13

some lines were not available, but the data do permit certain comparisons to be made. These are given in table 95. In the lines which have both the second and third chromosomes replaced, the difference between the X lines with and without the pod-X in the males is not significant, nor does the difference between the X lines and the ClB lines appear to deviate significantly. If the X lines with pod-X (in the males) and pod-2 are compared to the ClB lines pod-X, pod-2, there is no significant difference. Therefore it appears as if the addition of a nonpod-Y chromosome to the genotype of the females has no effect on the penetrance of podoptera.

But in these lines the genotypes of the females and males are different. Therefore a comparison of the males only should give a reliable determination of the effect of having a Y in the mother's genotype. Such a test was possible only for the lines having pod-2, replaced 3, in which the females from the X lines were $\overline{X}Y$ and the males were X^pY , and the ClB-line females were X^pX^p and the males were X^pY (table 96). Although the percentage values vary considerably there is no significant difference between the incidence of podoptera in X-line males and ClB-line males. Therefore it is probable that the penetrance of podoptera in the males is not affected by the introduction of a nonpod-Y into the genotype of the mother. However, the fact must be taken into consideration that there are strong pod factors in the Stubble and Hairless chromosomes, and that these in turn are enhanced by nonpod-X and -Y chromosome factors.

TABLE 95

COMPARISON OF INCIDENCE OF PODOPTERA H IN $\bar{X}$ AND CIB LINES IN WHICH THE pod-Y HAS BEEN REPLACED

Lane	Chromosomes	X replaced				X pod				χ^2
		Derivation of X and Y chromosomes	Total flies	pod		Derivation of X and Y chromosomes	Total flies	pod		
				♀	♂			♀	♂	
$\bar{X}$	2, 3 repl	♀ $\bar{X}Y$ ♂ $\bar{X}Y$	1,074	2	2	0 37	3	3	0 50	2 71
CIB	2, 3 repl	♀ XX ♂ XY	1,168	2		0 17	5	3	0 71	
$\bar{X}$	2 repl; 3 pod	♀ $\bar{X}Y$ ♂ $\bar{X}Y$	1,294	2	2	0 31	1	5	0 56	0 38
CIB	2 repl; 3 pod	♀ XX ♂ XY	1,161	1		0 08				
$\bar{X}$	2 pod; 3 repl	♀ $\bar{X}Y$ ♂ $\bar{X}Y$					3	2	2 44	23 40
CIB	2 pod, 3 repl	♀ XX ♂ XY	1,037	75	40	11 08	31	16	4 93	

When a pod-Y is introduced in the $\underline{X}$ genotype replacing the nonpod-Y, the results are considerably different. The degrees of penetrance of podoptera in similar $\underline{X}$ and CIB lines were compared (table 97). In this case the genotypes of the attached X females was always $\underline{y}Y^p$ and that of the males either X^pY^p or XY^p . The differences between the $\underline{X}$ and CIB lines without the pod-2 chromosome were statistically significant with the $\underline{X}$ lines having the highest

TABLE 96
COMPARISON OF INCIDENCE OF PODOPTERA II IN FEMALES AND MALES IN $\underline{X}$ X, 2
AND CIB X, 2 LINES

	$\underline{X}$ line X, 2			CIB line X, 2			χ^2
	Total flies	pod	Percentage	Total flies	pod	Percentage	$\underline{X}$: CIB
Total.....	205	5	2.41	953	47	4.93	2.47
♂.....	102	2	1.97	449	16	3.56	0.31

TABLE 97
COMPARISON OF INCIDENCE OF PODOPTERA II IN $\underline{X}$ Y AND CIB Y LINES HAVING
THE pod Y CHROMOSOME

	$\underline{X}$ lines			CIB lines			χ^2
	Total flies	pod	Percentage	Total flies	pod	Percentage	$\underline{X}$: CIB
Y3.....	696	9	1.20	1,257	3	0.24	6.29*
XY.....	494	11	2.21	1,236	6	0.48	8.86*
Y2,3.....	1,490	6	0.40	2,117	13	0.61	6.29*
XY2.....	751	52	6.92	1,078	146	13.54	17.79**
XY2,3.....	1,772	16	0.92	2,939	82	2.79	17.97**

* Probability between the 5 per cent and 1 per cent levels is indicated by an asterisk in this and the next table.

** Probability beyond the 1 per cent level.

incidence of podoptera. In the lines which had pod-2 the differences were again statistically significant, but in this case the CIB lines had the highest incidence of podoptera. However, the great differences in the last two groups may not be due to the Y chromosome but to the fact that the females in the CIB lines have both pod-X chromosomes. Therefore, in table 98, the females and males are compared separately. Again, except when the genotype was $Y^p2^p3^p$ there was a significant difference between the penetrance of podoptera in the $\underline{X}$ lines and the CIB lines, with the higher incidence of podoptera in the CIB lines. It is probable, therefore, that the pod-Y chromosome in the $\underline{X}$ lines is responsible for the reduction of penetrance of podoptera.

When the $\underline{X}$ nonpod-Y lines were compared to the CIB nonpod-Y lines, no significant difference was found, and thus the conclusion that the Y had no effect on pod expression, was permissible. But when the pod-Y lines were compared the difference was significant, and the conclusion was that the pod-Y had an inhibiting effect. An explanation of this discrepancy and the validity of the conclusions that the reduction of incidence of podoptera is determined

by the Y chromosome was tested by comparing the penetrance of podoptera in the different $\underline{X}$ lines. The statistics of this test are given in detail in tables 99 and 100 and are summarized in table 101 in the same way as they were for the CIB and pod lines in table 93. No significant difference was found between lines which had only an X or a Y: e.g., replaced to X; X to Y, or if the pod third chromosome was present; 3 to X, 3; 3 to XY, 3; X to X, 3; Y, 2, 3 to XY, 2, 3 except in two cases: 3 to Y, 3 had a higher incidence in the Y, 3 line, and Y,3 to XY, 3 had a higher incidence in the XY, 3 line. The XY group had a higher incidence of podoptera in XY and XY, 2 lines, but not in XY, 2, 3 or XY, 3 lines.

TABLE 98

INCIDENCE OF PODOPTERA H IN FEMALES AND MALES OF $\underline{X}$ AND CIB LINES WITH pod-Y

Line	$\underline{X}$ lines			CIB			χ^2 $\underline{X}$: CIB
	Total flies	pod	Percentage	Total flies	pod	Percentage	
♀ Y2.	331	21	6.34	1,161	128	11.02	5.45*
Y2,3.	1,469	10	0.68	2,108	20	0.95	0.73
XY2.	420	31	7.61	566	66	11.66	4.74*
♂ XY2,3. . . .	881	8	0.91	1,330	30	2.26	4.91*
Y2,3.	862	4	0.47	974	8	0.82	0.90

With the exception of the lines containing pod-3 the data agree with those from the CIB and pod lines. The highest incidence of podoptera is found in the lines with the greatest number of pod chromosomes. But the incidence of podoptera in the $\underline{X}$ lines is not as high as in the CIB and pod lines. The Y in the mother inhibits the expression of the podoptera phenotype to a greater or lesser degree depending upon the other podoptera chromosomes present. When the nonpod-X is with a nonpod-Y the results are the same as those when the nonpod-X is substituted for the pod-X in the CIB and Pod lines. If a pod-Y is present in the female genotype and either a pod-X or a pod-Y in the male, there is no increase in incidence of podoptera; but if a pod-Y is present in the female genotype and a pod-X and pod-Y in the male, the incidence of podoptera is inhibited to some extent but is less in the males than in the females.

This explanation does not take into account the deviations in the lines having pod-3. However, as 12 of the 21 podoptera were also Minute-like (table 102), the unexpectedly "high" incidence of podoptera is probably primarily the effect of the Minute-like factor in the third chromosome. If these data are compared to similar data from the CIB and pod lines, it is found that the incidence of Minute-like flies is no greater in the $\underline{X}$ lines, but the incidence of Minute-like podoptera was seven times greater.

The Minute-like podoptera flies appeared only in nonpod-2 lines, but with all combinations of pod-X and pod-Y chromosomes. Although the incidence of Minute-like podoptera is higher in the males than in the females, the most obvious explanation is that the Y (in the female genotype) enhances the ex-

TABLE 99
INCIDENCE OF PODOPTERA H IN DIFFERENT COMBINATIONS OF XY2 AND 3 CHROMOSOMES IN THE X LINES
(Data given only for groups which have a total of ten or more podoptera in combined lines)

Compared	Total flies	pod	Percentage	Total flies	pod	Percentage	x ²	Difference
repl to X.....	1,074	4	0.37	1,204	6	0.50	0.01	Not significant
repl to XY.....	1,074	4	0.37	494	11	2.21	10.67	Higher in XY
3 to X3.....	1,292	4	0.31	1,064	6	0.56	0.38	Not significant
3 to Y3.....	1,292	4	0.31	696	9	1.29	5.27	Higher in Y3
3 to XY3.....	1,292	4	0.31	1,070	2	0.19	0.62	Not significant
X to Y.....	1,204	6	0.50	1,121	3	0.27	0.30	Not significant
X to XY.....	1,204	6	0.50	494	11	2.21	8.86	Higher in XY
X3 to Y3.....	1,064	6	0.56	696	9	1.29	1.97	Not significant
X2 to XY2.....	2,205	5	2.44	751	52	6.92	5.14	Higher in XY2
X3 to XY3.....	1,064	6	0.56	1,070	2	0.19	1.12	Not significant
Y to XY.....	1,121	3	0.27	494	11	2.21	12.73	Higher in XY
Y3 to XY3.....	696	9	1.29	1,070	2	0.19	6.78	Higher in XY
Y2,3 to XY2,3.....	1,490	6	0.40	1,722	16	0.92	2.32	Not significant

TABLE 100
INCIDENCE OF PODOPTERA H IN FEMALES AND MALES IN THE X LINES (CONTINUED FROM TABLE 99)
(Data given only for groups which have a total of 10 or more pod in combined lines)

Compared	Sex	Total flies	pod	Percentage	Total flies	pod	Percentage	x ²	Difference
repl to XY.....	♀	477	2	0.42	226	9	3.98	10.37	Higher in XY
X to XY.....	♀	610	3	0.49	226	9	3.98	11.70	Higher in XY
X2 to XY2.....	♀	103	3	2.91	331	21	6.34	1.17	Not significant
X2 to XY2.....	♂	102	2	1.97	420	31	7.38	3.19	Not significant
Y2,3 to XY2,3.....	♀	628	2	0.32	841	8	0.95	1.32	Not significant
Y2,3 to XY2,3.....	♂	862	4	0.46	881	8	0.71	0.63	Not significant
Y to XY.....	♀	517	9	0.19	226	9	3.98	14.40	Higher in XY

TABLE 101
E₁ AND NONPOD CHROMOSOMES UPON THE EXPRESSION OF PODOPTERA H IN X LINES

	2d and 3d chromo- somes	X, X pod, Y pod			X, X repl, Y pod			X, X pod, Y repl			X, X repl, Y repl		
		Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂
X repl, Y repl....	repl 3	XY>repl	XY>repl	—	—	—	—	X=repl	—	—	per cent 0.37	per cent 0.42	per cent 0.34
		XY=repl	—	—	Y>repl	—	—	X=repl X=repl	—	—	0.31	0.38	0.26
X pod, Y repl....	2 repl 3	XY>X	XY=X	XY=X	—	—	—	per cent 2.44	per cent 2.91	per cent 1.97			
		XY>X	XY>X	—	Y=X	—	—	0.50	0.49	0.51			
X repl, Y pod....	2 2,3 repl 3	X	X	X	per cent X	per cent X	per cent X	0.56	0.21	0.87			
		XY=X	XY=X	XY=X	0.40	0.32	0.46						
X pod, Y pod....	2 2,3 repl 3	XY>X	XY>Y	—	0.27	0.19	0.33						
		XY<Y	—	—	1.29	1.23	1.34						
X pod, Y pod....	2 2,3 repl 3	per cent	per cent	per cent	per cent	per cent	per cent				Legend: = no significant difference. > greater than. < less than. repl replaced — numbers less than ten. x no data.		
		6.92	6.34	7.38	0.92	0.95	0.91						
		0.92	0.95	0.91	2.21	3.98	0.71						
		0.19		0.33	0.19		0.33						

Legend:
= no significant difference,
> greater than,
< less than,
repl replaced
— numbers less than ten,
x no data.

pression of podoptera, or the effects connected with the $\underline{X}Y$ combination are conducive to enhance podoptera expression of the third-chromosome Minute-like podoptera factor.

The results of tests in the $\underline{X}$ lines permit certain conclusions to be drawn concerning the effect of the Y upon the expression of podoptera in pod-H:

(1) The pod modifiers in the X and Y chromosomes are specific in their effect upon the chromosomes of their own stock; that is, $\underline{X}Y$ chromosomes enhance pod action when combined with Curly/Plum and with Hairless/Stubble, and they reduce incidence when combined with pod second and third chromosomes, whereas X^p and Y^p enhance expression of pod second and pod third chromosomes.

TABLE 102
INCIDENCE OF MINUTE-LIKE AND MINUTE-LIKE PODOPTERA FLIES IN THE $\underline{X}$
REPLACEMENT LINES OF pod-H

Line	Total flies	Total pod		M pod		M nonpod	
		♀	♂	♀	♂	♀	♂
$\underline{X}3$	1,292	2	2	1	1	2	..
$\underline{X} X3$	1,064	1	5	..	5	3	3
$\underline{X} Y3$	696	4	5	2	3	1	1
$\underline{X} XY3$	1,070	..	2	..	..	4	5
Total $\underline{X}$	4,122	7	14	3	9	10	9
Same ClB-pod.....	5,026	5	2	2	1	4	11

(2) The $\underline{X}Y$ combination or the Y chromosome in the female have enhancing effects when combined with the Minute-like pod factor in the third chromosome.

(3) The presence of a pod-Y chromosome in the mother inhibits the expression of podoptera in sons; in the daughters it cannot be determined whether the decreased penetrance is due to the presence of the Y in the mother's or in the daughter's genome. This effect is probably primarily on the pod action of the second-chromosome factor.

(7) *Collaboration of podoptera factors in the X and Y chromosomes with the second-chromosome factor in heterozygous condition.*—The effect of the pod-X upon dominance of pod was tested by comparing the incidence of podoptera in flies heterozygous and homozygous for the pod-X chromosome (table 103).

No significant difference was found for the expression of podoptera if the X is heterozygous or homozygous pod ($\chi^2 = 0.08$), therefore the homozygous pod-X factor does not have a higher dominance-enhancing effect on the heterozygous second-chromosome pod factor than the heterozygous pod-X factor.

But if the incidence of podoptera in the Curly flies is compared to that of the Plum flies, the difference is significant ($\chi^2 = 7.04$) when the X chromosome is homozygous pod, but is not significant when the X chromosome is heterozygous. These results would suggest that the pod-X factor may have a

dominance-enhancing effect upon the expression of the second-chromosome factor. But, as there is not a significant difference between the Plum flies heterozygous for pod-X and those homozygous for pod-X, the effect may be attributed either to the effect of the X chromosome or to the enhancing effect of the Plum chromosome.

TABLE 103

EFFECT OF HETEROZYGOUS AND HOMOZYGOUS pod-X UPON THE EXPRESSION OF pod-H IN FLIES HETEROZYGOUS FOR THE SECOND CHROMOSOME

X chromosome	2d chromosome	Total	pod	χ^2
X ^p X ^p	Pm/2 ^p	833	19	7.04*
	Cy/2 ^p	840	5	
	Total.....	1,673	24	
X ^p X	Pm/2 ^p	485	8	2.25
	Cy/2 ^p	481	4	
	Total.....	966	12	

TABLE 104

EFFECT OF pod-X AND pod-Y ON THE EXPRESSION OF PODOPTERA H WHEN THE SECOND CHROMOSOME IS HETEROZYGOUS

	Cy/pod-2		Pm/pod-2		Total		χ^2
	Total flies	pod	Total flies	pod	Total flies	pod	
X ^p Y ^p	468	1	400	5	868	6	1.24
X Y ^p	192	..	156	..	348	..	
X ^p Y.....	481	2	556	5	1,037	7	1.66
X Y.....	233	1	248	4	481	5	
Total.....	1,374	4	1,360	14			
χ^2	4.50						

In table 104 a comparable test is made for X and Y in males. The four combinations of X and Y were tested for their effect upon the expression of podoptera if the second chromosome is heterozygous. As is true for the females, the podoptera X had no dominance-enhancing effect on the factor in the second chromosome in the males. There is no significant difference in incidence of podoptera between X^pY^p and XY^p ($\chi^2 = 1.24$) and X^pY and XY ($\chi^2 = 1.66$).

The pod Y when alone appears to have an inhibiting effect upon the expression of podoptera if the pod second chromosome is heterozygous. No podoptera were found among 631 flies of the genotype XY^p (118 of these having all podoptera chromosomes except the X), whereas 5 podoptera were present among 630 flies with the XY chromosomes.

d. Podoptera M(3)124

A number of chromosome-replacement lines of pod-M(3)124 were bred in single-pair matings. The results are shown in table 105. The individual chromosomes produce a penetrance which is very small in comparison to the 20 per cent penetrance found in the homozygous stock. In the isolated X chromosome line (no. 5) penetrance of podoptera is 0.6 per cent. The X chromosome combined with the second chromosome produces 0.8 per cent

TABLE 105
ISOLATION OF pod-M(3)124 CHROMOSOMES
(For explanation see table 75)

No.	Chromosomes	+	pod ♀	pod ♂	Percentage of pod
1	♀ $\underline{X}$ ♂ 1, Y alternates.....	937	2	11	1.4
a	Even-numbered generations. .	730	..	6	
b	Odd-numbered generations.....	207	2	5	
2	♀ $\underline{X}$, Y ♂ Y.....	174	..	..	...
3	♀ $\underline{X}$, Y2 ♂ Y2.....	1,163	20	17	3.1
4	♀ $\underline{X}$ 2 ♂ 1,2 Y alternates.....	1,011	6	2	0.8
a	Even-numbered generations...	814	5	2	...
b	Odd-numbered generations.....	197	1	..	...
5	♀ and ♂ 1.....	801	1	4	0.6
6	♀ and ♂ 1,2.....	1,309	3	7	0.8
7	♀ and ♂ 1,3.....	1,148	3	7	0.9

penetrance (no. 6), and the X and third chromosomes combined, 0.9 per cent. Since the backcrosses (table 74) showed that the presence of the Minute mutant is essential for manifestation of the podoptera phenotype in pod-M(3)124, the low penetrance of nos. 5 and 6 may be due to the absence of the Minute mutant. On the other hand, the results of line 7 demonstrate that the presence of the Minute mutant alone does not suffice to produce a high incidence of podoptera. Other replacement lines containing the Minute mutant could not be made, because the $\underline{y}$ M(3)124 females were almost completely sterile. The highest penetrance in the whole group of replacement lines was obtained when the second chromosome and the Y chromosome of pod-M(3)124 were combined (no. 3). The Y chromosome alone (no. 2) produces little or no podoptera. In nos. 1 and 4, in which the Y of M(3)124 alternates between females and males so that in all even-numbered generations the males carry the pod-Y and in all odd-numbered generations the females carry it, the influence of the pod-Y on penetrance should become evident. But no such effect can be detected. Therefore it is quite possible that

the higher incidence in no. 3 is due to the action of the Hairless and Stubble chromosomes from the marker stock. The results obtained in chromosome-replacement lines of pod-M(3)124 indicate that the X, Y, second and third chromosomes of this stock may all carry pod factors which, when isolated from one another, produce a penetrance of podoptera which is only a small fraction of that found in the homozygous pod-M(3)124 stock.

TABLE 106
ISOLATION OF pod-K CHROMOSOMES
(For explanation see table 75)

No.	Chromosomes	+	pod ♀	pod ♂	Percentage of pod
1	♀ $\underline{X}$ ♂ 1, Y alternates.....	228	2	8	4.2
a	Even-numbered generations.....	38	1	5	
b	Odd-numbered generations.....	190	1	3	
2	♀ $\underline{XY}$ ♂ Y.....	669	5	4	1.3
3	♀ $\underline{XY}2$ ♂ Y2.....	458	12	21	6.7
4	♀ $\underline{XY}3$ ♂ Y3.....	890	..	6	0.7
5	♀ $\underline{X}2$ ♂ 1, 2 Y alternates.....	1,126	6	7	1.1
a	Even-numbered generations.....	713	3	4	
b	Odd-numbered generations.....	413	3	3	
6	♀ $\underline{X}3$ ♂ 1, 3 Y alternates.....	840	..	89	9.5
a	Even-numbered generations.....	210	..	14	
b	Odd-numbered generations.....	630	..	75	
7	♀ 1 ♂ 1.....	1,143	2	17	1.6 (3.2)
8	♀ 1,2 ♂ 1,2.....	646	1	8	1.4 (2.8)
9	♀ 1,3 ♂ 1,3.....	1,632	1	63	3.8 (7.6)

e. Podoptera K

In table 106 a number of replacement lines of pod-K are tabulated. It has been shown (table 72) that unless the second chromosome from pod-K is present in backcross segregants, the podoptera phenotype will not appear. The strongest podoptera action is therefore expected for the isolated second chromosome (no. 3 in table 106 if the presence of the Y is disregarded). The incidence of pod in the line is 6.7 per cent (as compared to about 20 per cent in the homozygous stock). In combination with the pod-X (no. 8) the second chromosome produced 1.4 per cent podoptera, which is about the same incidence as was found for the isolated pod-X (no. 7). This discrepancy may be due to two factors: (1) In nos. 7-9 very few pod females were found, since these are lines without attached X chromosomes (p. 92), therefore the incidence of pod in the males should be doubled for comparison with nos. 1-6. (2) In no. 3

the Y chromosome from pod-K is combined with the pod-2. It is therefore necessary to determine the effect of the isolated pod-Y (no. 2). The X chromosome and the autosomes are replaced in this line, the Y alone produces 1.3 per cent podoptera. If, however, the pod-Y and pod-3 are combined (no. 4) the incidence of pod is lower than that produced by the isolated pod-Y or by the pod-X and pod-3 combined (no. 9). The three lines (nos. 1, 5, 6) in which the pod Y alternates between generations also present a completely irregular picture. In the males the X together with the Y chromosome (no. 1 even-numbered generations) produced high incidence of pod in a very small sample of flies, the X alone had about as much effect as in no. 7. This would again point to a podoptera effect of the pod-Y chromosome, but nos. 5 and 6 are not in agreement with this assumption. The low incidence of podoptera in no. 5, i.e., in a line with the pod-2 chromosome, cannot be explained, and the high incidence in no. 6, apparently due to the presence of the pod-3 chromosome, does not confirm the estimation of the podoptera effect of these two chromosomes derived from the backcrosses.

The homozygous second chromosome of pod-K, which is expected to produce a high incidence of podoptera in lines 3, 5, and 8, has the expected effect only in line 3. In line 5 only a very small number of podoptera flies were obtained. The pod effect of the third chromosome, on the other hand, was greater than expected, especially in line 6. Therefore lines 5 and 6 were again studied for incidence of podoptera. The results are as follows:

	Line no. 5	Line no. 6
Normal.....	821	1,415
Pod..... ..	6	16
Spread wing.....	0	37

The spread-wing phenotype resembles podoptera class I, but the two effects can usually be separated. In the presence of Curly (in line 6), however, an error may have been made in classifying the wing effects. Thus, the actual incidence of podoptera in this line may be much lower than reported in table 106.

All the isolated chromosomes of pod-K seem to be able to produce the podoptera phenotype with low frequency, but the high penetrance of pod in homozygous pod-K must be based upon multiplicative rather than additive action of the individual factors.

C. ATTEMPTS AT EXACT LOCALIZATION OF POD FACTORS IN INDIVIDUAL CHROMOSOMES

a. *Tetraltera* and sex-linked factors

The foregoing facts made it obvious that an exact localization of pod or tet factors is difficult to accomplish. But some information could be gained. The best success should be obtainable with the sex-linked tet factors which have a reasonably good penetrance by themselves. In order to exclude the disturb-

ing action of the autosomes, a stock of selected tet was used in which second and third chromosomes but not X and Y were replaced, i.e., the tet X was isolated. Males of this constitution were crossed to y cv v f females, and the F₂ was checked for tet. Among a large number of flies the following tet flies were found:

tet	2 ♀	4 ♂
y cv v f tet		1 ♂
y cv v tet		1 ♂
y cv tet	1 ♀	2 ♂
y cv f tet		2 ♂
f tet		1 ♂

This shows at once the great difficulties encountered. As no females with two X chromosomes from tet can appear in this cross, no tet females are expected on the basis of the sex-linked factor. The 3 females actually found must therefore be due to action of autosomal factors, which could be factors in the neglected fourth chromosome or tet factors from the dominant marker stock present after isolation of tet-X (see above). Therefore, in the group of tet males some may be included which are tet individuals produced by action of autosomal factors, just as are those found among the females. The fact cannot be ignored that one of the presumably crossover or marker males which is decisive for the interpretation is in this group. The y cv v f tet male is an example. If this is a crossover male, tet must be situated beyond forked. If it is an autosomal tet male, the sex-linked factor might be located between v and f. But apparent crossover males might also be chance combinations of those autosomal factors with crossover X chromosomes. It must be kept in mind, however, that the probability for the appearance of tet phenotypes is much higher if the tet-X is combined with action of autosomal factors. Therefore, a tet male with markers has a greater chance to contain also the sex-linked tet factor. This means that a calculation based upon the assumption that the apparent crossovers are real ones has a certain chance to be nearly correct. But if tet were located beyond f, the probability of double and single crossovers with one break between f and tet would be a small one. Actually, 3 such males were encountered, which most probably are the autosomal counterparts of the 3 tet females with markers. If we make this assumption, one male in 8 is left as a crossover f tet, which places tet at about 12 units right of forked (of course with a large margin of error). In view of the unreliability of this procedure, it was not extended.

Better results were obtained with tet-100, which has two sex-linked tet factors whose combined penetrance was about 8 per cent in the original attached X tests. F₂ was made from tet-100 × y cv v f, which permits both females that are homozygous and males that are hemizygous for the sex-linked factors to be produced, with crossing over visible in the males only. The autosomal recombinations alone have very little effect in F₂ as compared with the sex-linked or combined effects (see above), and no foreign tet factors have been introduced (as they were in the experiment reported above where auto-

somes were replaced by dominant marker chromosomes). Thus the result of the experiment should be fairly clear. The F_2 data are:

1,054 ♀ ♂ +,	38 ♀	35 ♂ tet +
		3 ♂ y cv v f tet
		8 ♂ y cv v tet
		3 ♂ y cv tet
		2 ♂ y tet
		1 ♂ v tet

Here no tet females with markers could appear, as the cross was the reciprocal of the one above. Penetrance of tet was about 8 per cent, i.e., 16 per cent of those homo- or hemizygous for the sex-linked factor (in collaboration with whatever autosomal recombinations are present, assuming almost no action of the autosomes alone). The crossover values are in the correct order, but they are too small, which is perhaps explainable by the low numbers. The numbers show that the tet factors are situated about 6 units beyond forked. This is an average of the value for both tet factors, which cannot be studied separately. To prove that the tet factors had actually crossed over, the marked tet males were mated to attached X females. If tet had crossed over into the marker chromosome, the offspring should contain many tet males with the markers. If an autosomal combination were responsible for the y cv v f tet male, no tet flies would appear in F_1 . Thirteen such crossover males of all combinations were tested and showed the presence of the tet-X factor by producing many tet males with the respective markers. Therefore, the tet factors are located at a distance to the right of forked, which makes it possible that they are within the heterochromatin.

In view of the importance of this discovery further tests were made to secure a better localization.

1. (tet-100 × y cv v f) × tet-100

Result: 276 ♀ ♂ +,	19 ♀	20 ♂ tet +
		1 ♂ y cv v f tet
		3 ♂ y cv v tet
		3 ♂ y cv tet
		1 ♂ y tet
		1 ♂ v f tet
		1 ♂ f tet

Here, again, 15 per cent tet were found, i.e., 30 per cent for those homo- or hemizygous for tet-X. Since this was a backcross to tet, twice as many autosomal homozygotes were expected as in the foregoing example. The crossover group was similar to that in the earlier case. There were no females with markers, although in this group more purely autosomal female combinations may occur. The crossover value f-tet is 10 per cent, similar to the foregoing. If we add the data of these two tests, the distance of tet from f is 7.3.

2. (tet-100 × B)²

tet + 61 ♀	61 ♂
tet B 10 ♀	5 ♂

In this test, tet-100 was crossed to a Bar male; the penetrance of tet was 11 per cent. Half of both sexes in F_2 have the sex-linked tet factors, therefore penetrance for all flies with tet-X = 22 per cent (30 per cent obtained in example 1, 8 per cent expected without autosomal factors.) Since in this combination (F_2) the autosomal effect without the sex-linked factor is negligibly small (see table 59), the tet flies are almost completely due to the sex-linked factor in interaction with the autosomals in half of the flies. We may therefore calculate the crossover percentage directly from the data as a little less than 10.5 per cent. As Bar is located only 3 units to the right of the forked locus, the result is in agreement with the foregoing results.

3. (tet-100 $\times$ B) $\times$ tet-100

tet +	124 ♀	185 ♂
tet B	11 ♀	7 ♂

Here the penetrance of tet was 25.6 per cent, twice that of no. 2 as expected in a backcross to tet-100 because of the resulting higher autosomal homozygosity. Therefore, more of the tet flies may be of purely autosomal origin. But again, their expected number is relatively small as compared to those based upon interaction of X and autosomal factors. Thus, a direct calculation of crossover values will be almost correct. The value is 5.8. By adding the tests 2 and 3 we get a number high enough for us to calculate the position of the tet factors. The value is 7.7 per cent, which is almost identical with the no. 1 result (7.3). From this we deduce that the sex-linked tet factors are located in the section between f and bb; and, as a large heterochromatic block is located here, a localization within the heterochromatin is very probable.

We showed above that tet-100 contains two sex-linked tet factors (t and m) which a rough calculation put at 10 units distance from each other. There is no possibility of taking this into account in the present localization test. In a general way it would require that one factor is near f, the other near bb. But it would be very difficult to analyze this further. We found above an approximate distance of 12 units between f and the sex-linked factor t. Therefore, the m factor of tet-100 should be the one nearer to B. But avowedly the errors are too large to permit a more detailed analysis.

b. Autosomal factors

a. Tetraltera

A localization of the autosomal factors is more difficult because of the low penetrance of tet without the X-chromosome factor. Dominant markers are practically useless, because of their enhancing or pseudoallelic effect. Therefore, the recessive markers black and light (b 48.5, lt 55.0) were introduced into the isolated second chromosome. In tens of thousands of flies of the appropriate backcross only the following tet flies were obtained:

b lt	b	lt	+
62	6	15	6

This puts the tet factor about 13 units to the right of light, i.e., into the vestigial region. But this localization cannot be regarded as exact, because, apart from the large error, the numbers give a b-lt distance of 23 instead of 6.5, and far too many double crossovers b-lt-tet. Therefore, most probably something has modified the result, and it is as possible as not that tet is much nearer to light and the adjacent heterochromatin. In view of the difficulties with both dominant and recessive markers, no further attempts were made to find the exact locus.

The third-chromosome factors offer even greater difficulties. Dominant markers affect tet penetrance too much to be of use. A number of combinations with recessives were tested, but the low penetrance of the third-chromosome factor made only a few crossover flies available among tens of thousands of backcross individuals. The largest numbers were obtained with thread (43.2) and scarlet (44.0), namely, 16 th st, 3 +, 11 th, 1 st. This indicates a position of 19 units either to the right or to the left of the markers. The result is again very unsatisfactory.

β. Podoptera G

We found, above, that in pod-G the pod factors in the first and third chromosomes have effects so small that it is almost impossible to isolate them with desired markers. Thus, we concentrated mostly upon the second-chromosome factor which alone produced penetrance of pod of ± 3 per cent. The markers b lt (black 48.5, light 55.0) were put into this chromosome, and the backcross was made with the line of the Florida stock known to contain no pod factors. The results were (only pod flies):

b lt pod	+ pod	b pod	lt pod
92	17	2	2

These numbers do not permit a decision as to whether pod is right of light or not, or left of black. If pod were to the right of light, the location might be very near that of tet (light-tet = 13, light-pod = 19). Another combination was made with dp b lt pod. Again there were difficulties, first because the presence of dumpy seems to inhibit penetrance of pod, and second because pod class I cannot always be clearly distinguished in the presence of dp. Thus, the small numbers of pod flies obtained in a large experiment do not lend themselves to a numerical analysis. The numbers obtained were:

dp b lt	b lt	dp	dp b	+
6	6	3	1	4

The loci are dp 13.0, b 48.5, lt 55.0. The data indicate a location of pod to the right of light.

In view of these difficulties the dominant Star (S, 2-1.3) was crossed with extracted b lt pod and backcrossed. The result was (only pod flies):

b lt pod	S b lt pod	+ pod	S pod	lt pod	S lt pod	b pod
41	17	3	2	2	3	1

This agrees best with a location of pod to the right of lt at a distance of 8.7 units. Thus, we may add the two larger sets not involving the difficulties posed by dumpy and find pod at the locus 68.9, which is roughly the position we found also for tet. Thus, most probably both loci are located somewhere in the left quarter of 2R. Whether this is a heterochromatic region cannot be decided.

Tests for the third-chromosome factor were unsatisfactory. In view of the small penetrance of this factor, crossover individuals must be very rare, and we could not extract any with recessive markers. As dominant markers, Gl (Glued 41.4) and Bd (Beaded 93.8) were used. With Glued, more pod Gl flies than pod + appeared in RF₂; Gl, therefore, must be an enhancer of pod and cannot be used for localization. As regards Bd, we found subsequently that it is probably allelic to a pod factor (see below, p. 252). An RF₂ of Bd and pod-3 gave 23 pod +, 16 pod Bd. As Bd/+ has only about 3 per cent penetrance, such a number of pod Bd phenotypes would be impossible even with a high percentage of crossing over. Therefore it was assumed that Bd interacts with the pod factors in a special manner which makes it useless in localization experiments.

γ. Podoptera K

A crossing-over experiment with Lobe (2-72.0) was made to determine the locus of the podoptera factor in the second chromosome. The numbers obtained were: Lobe 383, ro-1 + 19, Lobe ro-1 7. Thus, assuming that ro-1 is at the locus of the pod factor, crossing over between Lobe and the pod factor was 3.5 per cent. The distance of 3.5 crossover units between Lobe and the podoptera factor represents only a tentative localization. Dominant markers are in general unsatisfactory for accurate localizations and, in this special case, rough eye and Lobe may not have been classified accurately in all instances, and the counts in this crossover class may therefore be too small. These data, however, are useful in determining the approximate region in the second chromosome in which the pod factor is located, which again is the same as in the other cases.

δ. Podoptera M(3)124

The action of the Minute without the rest of the third chromosomes of pod-M(3)124 and, at the same time, the action of the third chromosome from the Minute stock without the Minute mutant itself, were determined by a crossing-over experiment with recessive markers. Some F₁ males (Cy/pod-2; Sb/Minute) were mated to females of a stock which carried several recessive third-chromosome mutants (st sr e^s ro ca). In F₁, Cy Minute flies were selected to completely eliminate the second chromosome of pod-M(3)124. No attempt was made to eliminate the X chromosome from this stock. The females were then backcrossed to st sr e^s ro ca, and crossover individuals were selected from the offspring of this mating. Thus, lines with the Minute and different sections of the third chromosome from the Minute stock and others, again with different sections of the third chromosome but without the Minute, were established and tested for the appearance of podoptera (table 107). No podoptera was obtained with any of the segments except the one which in-

cluded the Minute, and the percentage of podoptera in that was very small. This is not unexpected, since all other podoptera factors had previously been eliminated from the stock. Only if the residual genome of the stock were gradually recombined with the lines having sections of the third chromosome could definite conclusions be obtained as to the location of the podoptera factor in one of these segments. The few podoptera obtained with the segment carrying the Minute do, however, indicate that at least the most important podoptera factor in the third chromosome is at or close to the locus of the Minute. Experiments on the relation of developmental delay to the manifestation of podoptera (see p. 152) support the assumption that one podoptera factor in pod-M(3)124 is actually identical with the Minute mutant.

TABLE 107
PODOPTERA EFFECT OF SECTIONS OF THE THIRD CHROMOSOME OF THE
STOCK pod-M(3)124

Section of M chromosomes without the M mutant	No. of cultures	No. of pod
st sr e ro.....	3	0
st sr e.....	2	0
sr e ro.....	4	0
sr e ro ca.....	6	0
e ro ca.....	2	0
Cultures with different sections containing the Minute mutant..	18	6 pod types I, II, III

3. ARE PODOPTERA AND TETRALTERA FACTORS INTERCHANGEABLE?

We found that the tet effect is phenotypically different from the pod effect in that in tet the whole wing is changed, in all transitions, into a haltere-like structure or into a wingleg. In pod, however, only the cubital part of the wing undergoes that transformation. Some of the extreme effects in both cases look identical, and frequently the classes I to III of tet and pod are indistinguishable. This does not, however, mean that tet is a pod effect which diverges only in the more extreme classes. Actually, we find in some individuals of class II a rather important difference, as described above. In tet the wing has a tendency to duplication of the *posterior* margin. In pod, however, there is a tendency to duplication at the *anterior* margin. Genetically this might have different meanings. The different types of determination in tet and pod may be based upon different main factors which are qualitatively different, and the penetrance might be controlled by additional factors which may or may not affect the pod-G and tet effects equally (as well as the other pod types; see the phenogenetic analysis). One case of positive collaboration of factors derived from different lines has already been mentioned in the section on the origin of tet-100 (see above) where a factor in the X chromosome of M(3)124,

which is also a pod stock, was introduced by crossing over into a tet X chromosome and increased the penetrance of tet in the presence of the tet autosomes and the sex-linked tet factor.

Of primary importance is the question whether one of the tet (pod) factors is qualitatively specific and the others contribute only to penetrance. If this is the case, only one of the isolated chromosomes should give the typical tet or pod effect, and the others would have no visible effect or would have an effect in which pod and tet could not be distinguished. Only the higher pod and tet classes would, as a rule, permit a decision, but some features of class II can be easily distinguished; and if, in a substitution experiment involving tet and pod stocks, a pod character, e.g., change of the cubital cell, had appeared, it would not have been overlooked. Actually, all tet flies from isolated chromosomes were really tet, not pod, and the same is true for pod. Thus, the specificity of the tet and pod-G action (and also that of the other stocks; see below) cannot be attributed to one or the other of the multiple factors. On the other hand, the M(3)124 sex-linked pod factor enhances penetrance and expressivity of tet without changing the tet type. But we must keep in mind that in such a combination tet factors are always present simultaneously and constitute most of the genome of the stock. The tet-100 X chromosome was studied further by combining the isolated second chromosome of pod-G with the X chromosome of tet-100, which is the most powerful tet agent known. The result was 425+, 12 females and 12 males pod, i.e., 5.4 per cent penetrance. Isolated first chromosomes of tet-100 had 3.5 per cent penetrance, and isolated second pod-G chromosomes 3.1 per cent female and 1.9 per cent male penetrance. The result of 5.4 per cent penetrance is not sufficiently higher above the level of action of tet-X alone to warrant a conclusion upon interaction of pod-G-2 and tet-100 factors.

These facts make it rather improbable that *all* tet factors are different from *all* pod factors—different in regard to different loci as well as to different alleles—although some of them must be specific for tet or pod. Thus, all the pod and tet factors may have a generalized action for homoeotic wing effect, but certain combinations may decide the type of wing effect, e.g., tet, pod-G, pod-K, etc. Since all these types segregate out of crosses with their specific morphological features, a general genetic background for the different types in the form of many specific modifiers is very improbable, and a specificity of one, or very few, of the factors must be assumed.

In a first group of experiments, tet and pod-G were crossed (table 108). An apparently positive result was obtained in no. 1 of the table, but an important question was posed immediately. Controls of non-tet stocks $\times$ tet or pod-G are normal, i.e., there is no dominance. If tet or pod flies appear in F_1 , it might mean allelism of tet and pod factors or a dominigene effect of the heterozygote. From the experiments with isolated chromosomes, we know the homozygous penetrance of each factor. The obtained penetrance is far below that of the second-chromosome factors, although it is in the neighborhood of the third-chromosome effect (compare tables 75, 77). This does not suggest that the F_1 result is due to allelism. A second possibility is that one or the other parent

introduces dominance enhancers. The whole body of facts is not in favor of this explanation. A third possibility is that two different factors might interact in heterozygous condition as if they were alleles, which we may call pseudoallelism (discussion in Part IV). Facts to be reported strongly suggest the latter interpretation.

In table 108 there is one cross (no. 7) in which the tet-100 X chromosome is present in the males. Isolated, it would produce 8 per cent penetrance. The high penetrance found (27 per cent) shows interaction with autosomal factors, although all are heterozygous (assuming that there is no allelism). This result

TABLE 108
CROSSES BETWEEN tet sel., tet-100 AND ISOGENIC pod-G

Cross	+	pod		tet		pod or tet		Penetrance
		♀	♂	♀	♂	♀	♂	
1. pod G × tet sel.....	919	..	2	1	..	..	..	0.33
2. F ₂ from no. 1.....	1,976	2	8	3	1	2	3	♀ 7 ♂ 1.2
3. (pod × tet) × pod.....	476	4	..	..	..	..	..	0.83
4. pod × (pod × tet).....	249	1	..	..	..	1	..	0.8
5. tet × (pod × tet).....	70	..	..	..	..	..	..	
6. (pod × tet) × tet.....	122	..	..	..	..	3	1	3.2
7. tet 100 × pod.....	180	..	..	..	28	..	..	♂ 27 per cent
8. F ₂ from no. 7.....	3,528	..	..	128	203	..	..	♀ 6.1 ♂ 10.5
9. Control (tet 100 × nonpod) ²	1,054	..	..	38	52	..	..	♀ 6.6 ♂ 9.1

seems to agree best with the foregoing conclusion upon pseudoallelism with the addition of the interaction of the sex-linked factor with the pseudoallelic autosomals. The phenotypically not-normal individuals were classified as pod, tet, and dubious. But only the tet type is really absolutely reliable, whereas the individuals classified as pod may belong partly to the dubious class. There is no doubt that reliable tet types appeared in F₁ no. 1 (the female tet was especially noted as being typical tet). As this F₁ tet fly should be a compound of the autosomal tet/pod factors, the tet action would appear to be dominant. All tet factors have a higher penetrance than pod factors which would parallel this dominance. The tet males in F₁ no. 7, were classified as tet types. But as only classes I-III were represented, this classification means merely that no pod types could be ascertained. It is regrettable that a decisive classification is here impossible because the X chromosome of tet-100 contains one factor each of pod-M(3)124 and tet origin, respectively. But one thing is certain, namely, the collaboration of this X chromosome with pod/tet autosomal compounds.

When F₂ no. 2 (pod × tet)² is compared to F₂ (pod × +)² with about 0.2 per cent penetrance and (+ × tet)² with 0.4 and 1.6 per cent for female and male, respectively, the penetrance is definitely close to that for (+ × tet)² alone, although a higher penetrance should be expected on the basis of F₁. On the other hand, the backcrosses 3 and 4 show the same penetrance as is known

for backcrosses ($\text{pod} \times +$) $\times$ pod . In this case only a slight increase would be expected. Real tet types were found in F_2 , but this does not furnish information on the genetic difference between the two types beyond that gained from F_1 .

F_2 no. 8 should be evaluated by comparison to an F_2 ($\text{tet } 100 \times +$)² no. 9. The penetrance is actually not so different from the control as would be expected on the basis of the F_1 result. Allelism for the sex-linked factor is excluded by the absence of tet females in F_1 . In this case definite tet and pod types could be distinguished among the segregants, namely, 5 females 8 males tet, and 6 females 3 males pod.

In view of these indecisive results, compounds were made from the lines in which the individual chromosomes had been isolated (see above). Table 109 contains the results.

In nos. 1-11 are tabulated the data for the isolated X chromosomes, which form a compound in the females only. It must be kept in mind that all contain the four dominant autosomal markers, which in themselves have a slight pod effect. Thus, not all the penetrance values below 1 per cent in the females should be considered. Only two compounds, nos. 10, 11, both with pod-G and pod-H, stand out as significant; the isolated X chromosomes both of pod-G and pod-H produce only very low pod penetrance (see tables 75 ff.), the compound shows an action above that of the individual homozygous actions. This looks as if the compound of the X chromosomes collaborates with the pod factors contained in the dominant marker chromosomes. (It will be noticed that no. 10 has a much higher penetrance than no. 11. These are reciprocal crosses, i.e., no. 10 with pod-H mother, no. 11 with pod-G mother. It will be shown in Part VI that pod-H exhibits maternal inheritance so that outcrosses with a pod-H mother show apparent dominance. This explains also the reciprocal difference, in this case, which is superimposed on the general result.) Nos. 12-21 contain the data for the second chromosome. Here all females are yY , which involves, therefore, the inhibiting action upon penetrance of the Y chromosome in the females in tet and must be taken into account. Pod-H has maternal inheritance and therefore has apparent dominance when used as mother of a cross, as seen, e.g., in the heterozygote with normal second chromosomes (no. 27), in which it has 2-3 per cent penetrance, whereas in homozygous lines the penetrance is 8-11 per cent. There is no case of absence of pod in the group nos. 12-21 as would be expected with nonallelic recessives (without pseudoallelism), and the compounds with pod-H as mothers show in the females a penetrance near that of the ordinary heterozygotes, and a much smaller one in the males. These are nos. 16, 17. But no 12, in which the father was pod-H, shows even higher penetrance, which then must be due to pseudoallelic interaction of the pod-K and H second-chromosome factors. The other compounds have, on the average, a much lower penetrance than either of the factors in homozygous condition; this is especially true in the males. Thus, allelism of the pod factors is excluded. The compound effect therefore must mean either that the compound increases dominance, or, that nonallelic pod factors interact as if they were partially allelic. Again, we may call this pseudoallelism. We shall show, below, that the existence of such pseudoallelism

ALLELISM TESTS FOR INDIVIDUAL CHROMOSOMES
(XK = X chromosomes from pod-K, etc.)

No.	Chromo- somes	♀	♂	Total flies	pod		% pod penetrance		Other chromo-somes
					♀	♂	♀	♂	
1	X	XK/XH	XH	282	..	1	..	0 7	♀ ♂ Cy/Pm Sb/H
2	X	XK/Xtet	Xtet	388	1	6	0 5	3 1	♀ ♂ Cy/Pm Sb/H
3	X	XG/XK	XK	582	2	13	0 7	4 5	♀ ♂ Cy/Pm Sb/H
4	X	XK/XM124	XM124	882	1	4	0 2	0 9	♀ ♂ Cy/Pm Sb/H
5	X	XH/Xtet	XH	523	..	1	.	0 4	♀ ♂ Cy/Pm Sb/H
6	X	XH/XM124	XM124	1,081	3	2	0 6	0 4	♀ ♂ Cy/Pm Sb/H
7	X	Xtet/XG	XG	1,964	2	1	0 4	0 2	♀ ♂ Cy/Pm Sb/H
8	X	Xtet/XM124	XM124	1,043	3	2	0 3	0 2	♀ ♂ Cy/Pm Sb/H
9	X	XG/XM124	XM124	880	1	.	0 2	.	♀ ♂ Cy/Pm Sb/H
10	X	XG/XH	XH	654	19	6	5 9	1 8	♀ ♂ heterozygous 2,3
11	X	XG/XH	XG	1,429	18	5	2 5	0 7	♀ ♂ heterozygous 2,3
12	2	2K/2H	2K/2H	276	4	1	3 0	0 7	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
13	2	2K/2tet	2K/2tet	155	4	1	1 3	.	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
14	2	2K/2G	2K/2G	272	4	2	3 0	1 5	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
15	2	2K/2M124	2K/2M124	350	..	1	..	0 6	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
16	2	2H/2tet	2H/2tet	349 (2 gyn)	3	1	1 7	0 6	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
17	2	2H/2G	2H/2G	339	3	2	1 8	1 2	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
18	2	2tet/2G	2tet/2G	873	6	1	1 4	0 2	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
19	2	2tet/2M124	2tet/2M124	353	..	2	..	1 1	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
20	2	2G/2M124	2G/2M124	530	1	4	0 4	1 5	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
21	2	2H/2M124	2H/2M124	371 (2 gyn)	5	2	2 7	1 1	all ♀ YY Sb/H; all ♂ foreign X, Sb/H
22	3	3K/3H	3K/3H	373	..	..	..	..	all ♀ YY Cy/Pm; all ♂ foreign X, Cy/Pm
23	3	3K/3tet	3K/3tet	138	..	..	..	..	all ♀ YY Cy/Pm; all ♂ foreign X, Cy/Pm
24	3	3K/3G	3K/3G	415	1	1	0 5	0 5	all ♀ YY Cy/Pm; all ♂ foreign X, Cy/Pm
25	3	3tet/3G	3tet/3G	934	4	2	0 9	0 4	all ♀ YY Cy/Pm; all ♂ foreign X, Cy/Pm
26	3	3G/3H	3G/3H	711	2	3	0 6	0 8	all ♀ YY Cy/Pm; all ♂ foreign X, Cy/Pm
27	Control	2/2H	2/2H	934	11	13	2 3	3 0	♀ YY Sb/+; ♂ Sb/+
28	Control	3/3H	3/3H	955	1	1	0 3	0 2	♀ YY Pm/+; ♂ Pm/+

between pod and many dominants is a typical feature of the pod reaction. The third chromosome of all pod lines has very little effect (see tables 75 ff.). (The third chromosome of pod-M(3)124 could not be isolated.) Thus, the smallness of the compound effect is not surprising. Otherwise, the results are of the same type as for the second chromosome. Altogether, we may say that there is no indication that the isolated pod factors are alleles, but they show an interaction of a type which can be described as pseudoallelism.

Another way of attacking the problem of the relations between the different pod factors is to combine by appropriate crosses chromosomes from different

TABLE 110

F₁ OF RECIPROCAL CROSSES OF pod-H AND pod-G INVOLVING ISOLATED CHROMOSOMES:
X^H/X^H; 2^H/2^H; H/Sb × X^G; Cy/Pm; 3^G/3^G

Phenotype F ₁	F ₁ ♀ X ^G X ^H , ♂ X ^H , mother pod-H			F ₁ ♀ X ^G X ^H , ♂ X ^G , mother pod-G		
	pod			pod		
	♀	♂	penetr. percentage	♀	♂	penetr. percentage
Cy H.....	8	3	6 9	6	2	2 2
Cy Sb.. . . .	3	1	2 4	..	..	...
Pm H.. . . .	3	..	1 8	8	1	2 5
Pm Sb.....	5	2	4 3	4	2	1 7
Total.....	19	6	3 8 (654)	18	5	1 6 (1429)
All with H.....	11	3	4 3	14	3	2 4
All with Sb	8	3	3 3	4	2	0 8
Control pod-H × Cy/Pm; H/SB.....	11	4	2 8 (539)			

χ² of totals experimental to control, 0.63, not significant.

pod lines with isolated chromosomes and to look for combination effects. Two groups of experiments were performed involving pod-G, pod-H, tet, and tet-100. Since pod-H appears dominant if used as mother, because of the maternal inheritance, a heterozygous combination is also of interest when this line is involved. Furthermore, reciprocal crosses should be kept separate because pod-H shows the maternal effect. Table 110 shows the F₁ results of crosses involving pod-H and pod-G. The crosses are: ♀ X^H/X^H; 2^H/2^H; H/Sb × ♂ X^G; Cy/Pm; 3^G/3^G and reciprocal.

In F₁ all females are compounds of the two X chromosomes, the males being X^H and X^G, respectively, and the autosomes are heterozygous compounds with one of the markers. It should be remembered that the marker stock contains low pod factors and that Hairless (H) especially acts like an allele or enhancer of a third-chromosome pod factor. Sb also acts like a pod factor. In view of the maternal inheritance of pod-H we expect higher penetrance in the left column, which looks like dominance of pod-H. But with pod-G as mother—the right column—we do not expect pod unless the marker stocks combine alleles or pseudoalleles of the pod factors, or the heterozygous pod-H and pod-G factors act as pseudoalleles. The rather high penetrance in the right

column, with no clear differences in regard to the markers, again favors the interpretation as pseudoallelic effect. With pod-H as mother, the penetrance is considerably higher than in the controls, although the difference is not significant.

Table 111 contains the results for the new recombinations extracted subsequently to F_2 . It shows that the highest penetrance is produced in combination of 2^H with the third-chromosome markers, which again is in conformity with F_1 . The 3^G has only a small effect (not distinguishable from a possible X-chromosome effect) and does not show a clear recombination effect with 2^H . The maternal effect visible in F_1 has completely disappeared, as expected in

TABLE 111
EXTRACTED RECOMBINATIONS IN F_2 ff. FROM F_1 TABLE 110
(X- Chromosomes All Possible Recombinations of X^H and X^G)

Chromosomes	Maternal line H				Maternal line G			
	Number	pod			Number	pod		
		♀	♂	penetr.		♀	♂	penetr.
2^H , 3marker	1,186	41	29	5.90	1,156	63	26	7.60
2marker, 3^G	1,018	2	1	0.29	1,295	3	4	0.54
2^H , 3^G	1,554	37	23	3.85	1,917	53	31	4.38
2marker, 3marker	1,556	20	8	1.80	784	10	6	2.16

maternal inheritance. Actually, all values are somewhat higher in the maternal pod-G line. There is no clear indication that pod-H and pod-G factors can collaborate as typical multiple factors.

Another experiment was made with tet, tet-100, and pod-G, with the intensive action of the X-chromosomal tet factors especially in view. As the second chromosome of pod-G contains the main pod factor, a combination with the powerful tet-100 X chromosome should show whether both can collaborate. No. 10 in table 112 contains this combination, which should be compared with the isolated tet-100 X chromosome and the combination tet-100-X, tet-2. The result is actually almost identical with that of the control, which means that the tet-100 X chromosome interacts with the second chromosome of pod-G. Unfortunately, the conclusion is not reliable, because the tet-100 line had undergone changes (reported above, p. 197) which resulted in a much lower effect of the X chromosome than had before been registered, and, as it seemed, the presence of different kinds of X chromosomes (see table 77 for isolation of tet-100 chromosomes). Standard tet, however, shows an unequivocal result: the combination (no. 5) has an effect which is not very different from that of the tet-X chromosome alone, and only half of tet-X, tet-2, thus indicating that the tet-X does not collaborate with pod-2. The same situation is visible for the combination tet-X, pod-3 (no. 6). The penetrance is comparable to that of tet-X alone and is considerably below tet-X, tet-3. The combination tet-2, pod-3 is not very different from tet-2, tet-3, but as the third

chromosome alone has only a very slight effect, this does not mean much. Finally, there is the combination of tet-X, tet-2 and pod-3. The low penetrance found excludes an interaction with pod. Altogether, we may say that the pod-G and tet chromosomes cannot replace each other in producing combination effects. In view of these negative results with the best-suited lines, no combinations with the other lines were made.

TABLE 112
COLLABORATION OF tet AND pod-G FACTORS

Combination	Total flies	pod		Percentage of pod	
		♀	♂	♀	♂
1. $\bar{X}$ Y pod-2, tet-3.....	811	8	25	1.9	6.0
2. $\bar{X}$ Y tet-2, pod-3.....	1,105	3	6	0.6	1.1
3. pod-X, tet-2.....	547	3	3	1.1	1.1
4. pod-X, tet-3.....	1,129	3	..	0.5	...
5. tet-X, pod-2.....	1,060	20	31	3.6	5.6
6. tet-X, pod-3.....	1,208	6	28	0.9	4.5
7. pod-X, 2, tet-3.....	1,329	7	4	1.0	0.6
8. pod-X, 3, tet-2.....	109	1	1	1.8	1.8
9. tet-X, 2, pod-3.....	1,176	16	18	2.6	3.0
10. tet-100-X, pod-2.....	405	11	12	5.1	5.6

TABLE 113
TEST FOR ALLELISM OF pod-G AND pod-M(3)124

Generation	Mating	Total flies	Percentage pod M	Percentage pod not M	Total percentage pod
F ₁	pod-G × pod-M(3)124.....	1,778	0.2	0.1	0.3
F ₂	pod M × M.....	95	2.1	...	2.1
F ₂	M × M.....	1,259	1.2	0.6	1.8

Since the replacement experiments showed that pod factors, when isolated, have a different pod action than when combined in the stock, the following data on the interrelation of podoptera stocks are added to the allelism tests. In the experiments to be reported, different inbred podoptera stocks were crossed, and the F₁ and F₂ manifestation of the podoptera phenotype was studied.

1. *Podoptera G* and *podoptera M(3)124*.—Penetrance of podoptera in F₁ is shown in table 113. The result suggests that the primary factors in the two stocks are not allelic but may be pseudoallelic.

From one of the rare podoptera flies obtained in F₁ (3 females pod Minute, 2 males pod not Minute), an F₂ was bred (table 113). In the offspring of this mating the incidence of podoptera was 2.1 per cent. Twelve hundred and fifty-nine F₂ individuals from matings of not podoptera Minute parents were classified. The data are also presented in table 113. The percentage of podoptera with Minute is lower than the comparable penetrance values obtained

after outcrossing to Florida (see table 64), but it is higher without Minute (0.6 per cent as compared to 0.1 per cent). The total penetrance is the same as in F_2 with the Florida stock. Thus, the podoptera factors of pod-G and pod-M(3)124 are not allelic and cannot be substituted for one another in homozygous condition.

2. *Podoptera G and podoptera K*.—In reciprocal crosses in F_1 (table 114) between these two stocks, podoptera was found with a frequency of 0.8 per cent in a mating in which the female was phenotypically pod from pod-G, a percentage which agrees with that of the crosses already reported. In the other mating, no podoptera was found. In F_2 of both types of crosses (table 114), incidence of podoptera was about 1 per cent. These results show that there is no allelism or collaboration of pod factors in the two stocks.

TABLE 114
TEST FOR ALLELISM OF pod-G AND pod-K

Generation	Mating	Total	Percentage of pod
F_1	pod-K $\times$ pod-G	495	...
F_1	pod-G (phenot. +) $\times$ pod-K	816	...
F_1	pod-G (phenot. pod) $\times$ pod-K	470	0.8
F_2	(pod-G $\times$ pod-K) ²	1,638	0.7
F_2	(pod-K $\times$ pod-G) ²	745	1.3

3. *Podoptera M(3)124 and podoptera K*.—Results obtained in reciprocal crosses of pod-M(3)124 and pod-K are presented in table 115. In F_1 of the cross pod-M(3)124 $\times$ pod-K, podoptera in combination with the Minute appears in both sexes with a penetrance of about 3 per cent. It is also found in very low numbers without the Minute. The incidence of podoptera (3 per cent in the females, 4 per cent in the males) is considerably lower than in either homozygous pod-M(3)124 or pod-K (about 20 per cent in both stocks). Therefore the podoptera factors in the two stocks do not appear to be alleles. On the other hand, there is collaboration between the heterozygous factors, since a very low number of pod flies is obtained in F_1 when either of the stocks is outcrossed to a nonpodoptera stock.

The great preponderance of Minute podoptera individuals indicates that the Minute mutant is very important in producing the podoptera phenotype. There are, furthermore, as many pod males as pod females. Since the females are not attached X, no pod females are expected on the basis of the pod-K factors. The rough-eye and notched-wing phenotypes, which are always found together with podoptera in the pod-K stock, do not appear in F_1 with pod-M(3)124. All these facts suggest that the Minute mutant collaborates with heterozygous pod factors in the other chromosomes (and possibly in the third chromosome of pod-K) to produce essentially the podoptera reaction characteristic of pod-M(3)124. In F_2 (table 115) podoptera phenotypes are found with and without Minute. Penetrance of pod is much higher than in F_2 with nonpodoptera stocks (table 62, 63, 64).

TABLE 115
TEST FOR ALLELISM OF pod-M(3)124 AND pod-K

Gener- ation	Mating	Total	ro-l		pod		pod M		pod ro-l M		pod ro-l		Total percentage pod	
			♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
F ₁	pod-M(3)124 × pod-K.....	751	..	..	2	2	22	28	..	..	..	..	3 1	4 0
F ₂	pod M × pod M....	563	..	14	7	4	49	31	..	3	..	8	18 5	25 2
F ₂	pod × +.....	228	..	..	13	13	..	..	..	..	..	13	11 2	22 5

Segregation in F_2 (no. 2, table 115) of the different types characteristic of each stock makes possible an estimation of the nature of the collaboration of the pod factors. Again, there are no pod-rough-eye or rough-eye females. Rough eye (ro-l) (with and without pod) was found in 10 per cent of the F_2 males. This agrees with F_2 values found after outcrosses with nonpodoptera stocks. The rough-eye and notched-wing phenotypes are therefore specific features of pod-K and are not affected by collaboration with factors of pod-M(3)124. The incidence of podoptera with rough eye was about 4 per cent, which is considerably higher than that found after outcrossing with nonpod stocks. Therefore it must be concluded that the pod factor in the second chromosome of pod-K does collaborate with the pod-M(3)124.

Flies which were both podoptera and Minute appeared in 12 per cent of the males and in 17 per cent of the females. These values approach incidence of pod in the homozygous pod-M(3)124 stock, and confirm the F_1 results by showing again that the Minute mutant collaborates very successfully with podoptera factors of pod-K.

There are also some podoptera flies (2 per cent) without the Minute mutant and without rough eye—meaning that they do not carry the homozygous second chromosome of pod-K (no. 2, table 115). The incidence of this group, as well as that of pod ro-l, was increased to 11 per cent (no. 3, table 115) in an F_2 podoptera $\times$ normal, although none of the F_1 flies were Minute. Even without the Minute mutant, the podoptera factors of pod-K and pod-M(3)124 collaborate to produce a relatively high incidence of podoptera.

4. THE Y CHROMOSOME

In the course of the genetic analysis we met repeatedly with facts which pointed to an influence of the Y chromosome upon the pod phenotype. This was especially clear in the case of pod-K. Therefore, the data on the action of individual chromosomes were arranged so that possible Y chromosome effects could be analyzed (see tables 75 ff). In addition, experiments were made in which extra Y chromosomes were introduced into some of the pod stocks.

A. PODOPTERA G

It is characteristic for pod-G that, when the flies are bred under standard conditions, the sex ratio of phenotypically pod flies is rather high in favor of females. In table 116 are assembled the data for different types of breeding and mating isogenic pod-G. There is no apparent correlation between the variability of the result and the types of mating made. All groups with large numbers show high sex ratios in favor of females; therefore, we may assume that the few low ratios that were obtained were based upon errors of sampling, and that the average ratio 2.2 : 1 represents the true mean. The same ratio had been found for nonisogenic lines. This ratio may be explained in different ways. It could be due to sex-linked lethals. This explanation can be excluded in the isogenic and subsequently highly inbred line. The presence of lethals would be reflected in the total sex ratio (i.e., + and pod phenotypes), but this is normal. The second possibility is that the character is a sex-con-

trolled (limited) one, which means that in the female developmental system the homozygous factors for pod expression succeed better in controlling development of the pod phenotype than they do in the male system, which differs from the female in many respects, such as in the rate of development. In support of this interpretation, it may be mentioned also that the higher classes of effect are rarer in males (Goldschmidt, 1945). The experiment with

TABLE 116
SEX RATIOS OF ISOGENIC pod-G, ONLY pod PHENOTYPES
(Total sex ratio is normal)

No.	Line	Matings	pod		♀ : ♂
			♀	♂	
1	5 gener. inbreeding.....	Diverse	55	34	1.6
2	13 gener. inbreeding line 14370	Brother-sister pod × pod.	234	86	2.7
3	13 gener. inbreeding line 14370.....	Brother-sister pod × +....	117	52	2 3
4	13 gener. inbreeding line 14370.....	Cousin pod × pod.....	33	20	1.7
5	13 gener. inbreeding line 14370.....	Cousin pod × +.....	31	11	2.8
6	13 gener. inbreeding line 14273.....	Brother-sister pod × pod .	239	110	2 2
7	13 gener. inbreeding line 14273.....	Brother-sister pod × +...	136	49	2 8
8	13 gener. inbreeding line 14273.....	Cousin pod × pod.....	64	32	2.0
9	13 gener. inbreeding line 14273.....	Cousin pod × +....	9	10	0.9
10	13 gener. inbreeding line 14349	Brother-sister pod × pod.	92	45	2 0
11	13 gener. inbreeding line 14349.....	Brother-sister pod × +....	69	46	1.5
12	All.....	All.....	1,024	461	2.2

extra Y chromosomes (below) may sustain this interpretation. The third possibility is that the Y chromosome has an inhibiting effect upon penetrance of pod, either by the generalized action of the heterochromatin of the Y, or by the presence of a specific pod inhibitor in the Y chromosome. To test these possibilities, the data contained in the series of chromosomal replacements (tables 75 ff.) are now tabulated so that the respective X and Y combinations become evident (table 117). In this table all broods with pod-X in both sexes are comparable to pure pod-XX and -XY except for the origin of the Y, which had been found to be irrelevant in pod-G. This is group I, which has the same sex ratio as the controls (2.3 vs. 2.2). We have also the group in which neither sex contains the pod-X and the females have an extra Y. These are nos. 4, 5, 6. Together they contain 74 ♀ 54 ♂, i.e., a ratio of 1.4. The combined penetrance

of this group is ♀ 1.0, ♂ 0.8. This shows that in the absence of the sex-linked pod factor the extra Y chromosome in females inhibits the pod action (we had found before that the pod-X has very little pod action). This inhibition is strongest in the presence of the third-chromosome factor, weakest with the powerful second-chromosome factor. The third group contains females having no pod-X but having an extra Y, and males carrying a pod-X (nos. 2, 3, 7). Here we find 49 ♀ 80 ♂, and the penetrance in this group is ♀ = 1.6 ♂ = 2.7. An increase in male penetrance in the presence of the X factor relative to that of the females without this factor is possible, but not so much as to change the normal sex ratio of 2.2 : 1 into 1 : 1.7. Thus, we notice again an inhibiting action of the extra Y upon the autosomal pod effect in the females.

TABLE 117

SEX RATIOS IN pod G IN RELATION TO THE Y CHROMOSOME

(Chromosomes not mentioned are those derived from y Cy/Pm H/Sb, including the Y. Thus, Y means Y from pod; if no X is mentioned, it means foreign X, i.e., in ♀ X.)

No.	pod chromosomes		pod		Ratio ♀ : ♂	% penetr.		n
	♀	♂	♀	♂		♀	♂	
1	XX (2 or 3)....	X (2 or 3).....	63	27	2.3	1 0	0 4	6,351
2	Y2.....	XY2.....	31	42	0.7	1.2	1.7	2,512
3	Y3... ..	XY3.....	11	33	0 3	0 8	2 6	2,525
4	Y2.....	Y2.....	51	25	0.2	2 0	0.9	2,642
5	Y3.....	Y3.....	13	23	0 6	0 5	0 9	2,605
6	Y.....	Y.....	10	6	1.7	0 5	0.3	1,922
7	Y.....	XY.....	7	5	1.4	0 8	0.6	897

Does this also mean that the low penetrance of pod-XY males as compared to pod-XX females is based upon an inhibiting effect and not on a sex-controlled condition? The next group of experiments is concerned with this problem, as well as with the effect of extra heterochromatin in general.

For our use in introducing extra heterochromatin into pod flies, Dr. H. J. Muller kindly furnished a stock. In this stock, the long arm of the Y chromosome (Y^L) is attached to two small portions of the X, one containing the $+^y$ locus (normal allele of yellow) and the other being a piece including the spindle-fiber attachment. Thus, these combined portions of the X and Y segregate as a unit. As $+^y$ suppresses homozygous yellow, the additional marking of the normal X chromosomes with homozygous yellow permits distinguishment of the individuals carrying the extra Y segment, because these flies are not yellow. (There are also crossover inhibitors and markers serving to distinguish classes.) Females of this stock with Y^L (phenotypically not yellow) were mated to pod-G males which contain yellow and white—position effects of $In(1)y^{pxbl}$ and $In(1)w$ —and not-yellow F_1 females were backcrossed to pod-G males. The resulting yellow flies, which do not contain Y^L , serve as controls for the not-yellow segregants which have the extra Y^L . In order to introduce additional Y material, a stock was made in which homozygous pod (isolated second

chromosome) is combined with yY in the females (the attached X containing yellow), and the males are marked with yellow white. Such females crossed with F_2 not-yellow males from the former cross, containing the extra Y^L in addition to a normal Y (males with Y^L alone are sterile) produce females and males with YY^L or YY . Table 118 contains the results of the two experiments.

The first cross seems to show that the addition of Y^L to a pod female does not reduce penetrance as did the addition of a whole Y. This might, at first sight, mean that the short arm or the entire Y is required for the inhibiting effect in the female. But we must not forget that females in this case have also

TABLE 118
Pod-G WITH EXTRA Y AND CONTROLS SEGREGATING SIMULTANEOUSLY
(yellow = controls, pod not yellow contains Y^L)

Cross	yellow		not yellow		pod yellow		pod not yellow	
	♀	♂	♀	♂	♀	♂	♀	♂
1. ($yy Y^L$ ♀ × pod y ♂) × pod y ♂	1,992	1,460	905	1,065	28	8	14	24
2. yY pod ♀ × $y YY^L$ pod ♂	1,238	1,224	557	470	13	17	10	27
3. Penetrance pod no. 1	. .	. . .	. . .	. . .	1 36	0 55	1.52	2.58
4. Sex ratio pod no. 1	. . .	. . .	. . .	. . .	3 5 ♀ : 1 ♂		1 ♀ : 1.71 ♂	
5. Penetrance pod no. 2					1 04	1.37	1.76	5.43
6. Sex ratio pod no. 2					1 ♀ : 1 3 ♂		1 ♀ : 2.7 ♂	

the pod-X chromosomes, whereas in the case discussed above they had only the autosomal pod factors. Actually, in the present experiment the female penetrance is slightly higher than in the controls. If we assume that, as in the yY case, penetrance was decreased by the extra Y^L in the group without homozygous pod-X (half of the flies) the actual increase in the other half with homozygous X more than compensates the decrease.

In the male the extra Y^L does not only counteract whatever causes the low male penetrance but actually enhances male penetrance to almost five times that of the control. There are two possible explanations of this result as indicated above: (1) The normal low penetrance of pod in the male sex has nothing to do with the Y chromosome but is a developmental consequence of the general differences between female and male differentiation (sex-controlled difference). The extra heterochromatin changes the male developmental pattern into that characteristic of the female, or goes even further and changes it into one still more favorable for pod penetrance. (2) The Y chromosome contains an inhibitor of pod penetrance (which might mean nothing but an inhibiting action of the heterochromatin) which acts as well in XY females as in XY males. Since an additional Y^L in the males reverses the inhibiting effect into an enhancing one, we could assume a dosage relation roughly com-

parable to that of the bobbed alleles. Here Stern (1929) showed that an increase in the number of alleles (1-4 doses) decreased the *bb* effect (shortening of bristles), and thus produced an approach toward normal bristles. In the present case this would mean that two *Y* inhibiting effects would be more normal than one, i.e., would produce less inhibition, just as 4 *bb* mutant loci reduce the bristles less than two. In view of the fact that male penetrance in the presence of the extra Y^L is not less inhibited but is greatly enhanced, the alternative, no. 1, seems more probable.

Experiment 2 is not directly comparable with no. 1. In no. 1, females and males have the same genetic composition as regards pod autosomes and X

TABLE 119
SEX RATIOS OF tet FLIES

No.	Combination	tet		Ratio ♀ : ♂	Penetr. tet
		♀	♂		
1	tet-100.....	112	105	1.1	100
2	tet high selection.....	252	304	0.8	70 and more
3	tet extracted from outcross, with homozygous X.....	221	283	0.8	28-61
4	Another series of same.....	467	500	0.8	
5	Extracted low-penetrance tet only autosomal.....	171	273	0.6	2-17, m = 8.2
6	$F_2 (+ \times tet)^2$, i.e., ♂ only with X ^{tet} ...	33	171	0.19	
7	$F_2 (tet \times +)^2$, i.e., ♀ ♂ with X ^{tet}	21	41	0.5	
8	$(tet-100 \times B)^2$	71	66	1.1	100

chromosomes. In the second experiment the not-yellow males all have the pod-X chromosome (marked *y w*) and the *Y* and Y^L (some may have two *Y*'s). Therefore, the same penetrance is expected as in no. 1, and since the pod-X chromosome does not segregate here as it does in no. 1, nearly twice the penetrance should be found. The results approximate this expectation. The not-yellow females are $\underline{X}Y^L$ and XY^LY^L . In the absence of the X pod factor, lower penetrance is expected, but that actually found is so low as to suggest again the inhibiting action of *Y* upon the autosomal pod factors in the female. Whether the second *Y* reverses this action or not cannot be judged from the available data.

B. TETRALTERA

The sex ratio of phenotypically tet flies is the opposite of that of pod-G. In tet the male is the preponderant sex among the tet types. In table 119 we have assembled the relevant data which are consistent and clear. (It should be remembered that tet has a powerful sex-linked factor.) In order to check upon the average sex ratio (of tet phenotypes) we have tabulated different lines, all of which are genetically comparable. No. 2 is a homozygous highly selected line, i.e., homozygous for all autosomal and sex-linked sex factors, as the penetrance indicates. No. 3 is a line extracted from outcrosses with high

penetrance, homozygous for the sex-linked factors although not homozygous for all autosomes. No. 4 is similar, taken from a different set of experiments. No. 5 is an extracted low-penetrance line without the sex-linked factors. No. 7 is an F_2 from an outcross in which one-half of both females and males contain the sex-linked factors in addition to identical autosomal recombinations, whereas no. 6 produces only one-half males with the tet X, and no females. All of these groups should have the sex ratio characteristic of tet, and it is approximately identical, ranging from 0.6 to 0.8. (No. 7 with 0.5 has a larger error because of the small number.) There remain no. 1, tet-100, with an approximately normal sex ratio, and no. 8, in which the segregating tet-100 have the

TABLE 120
Tet WITH EXTRA Y AS IN TABLE 118 FOR pod-G

Cross	yellow		not yellow		tet yellow		tet not yellow	
	♀	♂	♀	♂	♀	♂	♀	♂
1. (yy Y ^L ♀ × tet y ♂) × tet y ♂.....	2,253	1,842	1,305	1,078	126	178	162	374
2. y Y tet ♀ × y YY ^L tet ♂..	534	634	820	216	8	247	21	185
3. Penetrance tet no. 1.....					5.29	8.81	11.04	25.76
4. Sex ratio tet no. 1....					1 ♀ : 1.41 ♂		1 ♀ : 2.31 ♂	
5. Penetrance tet no. 2.....					1.48	28.1	2.5	46.25
6. Sex ratio tet no. 2.....					1 ♀ : 31 ♂		1 ♀ : 8.81 ♂	

same sex ratio as is found in parental tet-100. It is probable that this difference between tet and tet-100 is due to the fact that the highest grades of expressivity (class V, bilaterals), which are more frequent in tet-100 and also more frequent in males, are less viable than the lower grades (tet-100 has a very low viability).

The data for the effect of an extra Y upon penetrance in females have already been given above (table 64). They show clearly that penetrance in females, in the absence of the sex-linked tet factor and with an extra Y, is much reduced, as was also the case in pod-G. For a further analysis an experiment parallel to that reported for pod-G was performed with Muller's extra Y^L stock. For this experiment it was necessary to put yellow into the tet X chromosome (crossveinless was also present) and to select a tet line with this marked X chromosome for homozygous autosomal tet factors. This line furnished the males for the experiment. For the second experiment yY (attached X marked with yellow) was introduced into the tet line and was followed by selection of autosomal tet factors.

The results are presented in table 120, arranged like table 118 for pod-G. In no. 1 the controls (tet yellow) show the typical sex ratio for tet with male preponderance (0.7). In the presence of Y^L the penetrance has been doubled in the females and tripled in the males, both of the same genetic constitution

in regard to tet factors. (Half of the flies have the tet-X homo- or hemizygous.) Thus, the extra Y fragment enhances penetrance in females as well as in males in the presence of the sex-linked tet factor. In the second experiment the control females are yY and they have the low penetrance reported above for females without the sex-linked factor. In addition, Y acts here as an inhibitor, as mentioned above. The males, however, are partly XY, partly XY^L , and perhaps XY , and they show the same high penetrance as no. 1. (Note that all males have here the tet-X, whereas in no. 1 only one-half of them had it.) The not-yellow females have a higher penetrance than the yellow ones, a result which again must be due to the additional Y^L fragment. Thus, tet behaves exactly as pod-G but for the normal preponderance of males, and the discussion of the factors for pod-G applies also to the present facts.

TABLE 121

Cross	yellow	not yellow	pod yellow		pod not yellow	
			♀	♂	♀	♂
$(yy Y^L \times \text{pod } y) \times \text{pod } y$ Penetrance of pod.	854	817	2 0 5	12 2 7	25 5 8	28 6 4

C. PODOPTERA H

The effect of the Y chromosome upon penetrance of pod-H is still different and is rather complicated. A detailed discussion of the interaction of X, Y, and the autosomes was given on p. 206.

An experiment with extra Y chromosomes, similar to that for pod-G and tet, has thus far not been feasible, because the introduction of the proper markers and the attempt to extract a marked pod-H line of sufficient penetrance did not succeed.

D. PODOPTERA K

Following the procedure described above (p. 240), the Y^L was introduced into pod-K flies. The combination with yellow was obtained by selecting yellow pod males which occurred in the pod-K stock because of disjunction of the attached X chromosomes in the females. The new pod line, pod-K yellow, had, however, a rather low incidence of podoptera. Therefore, the results in table 121 cannot be compared with results of other experiments. In the control group (yellow flies) the males had 2.7 per cent pod. No pod was expected in the females, since they do not have attached X chromosomes. Actually, 2 pod females were found; it is not possible to explain this fact without further assumptions. If the controls are compared to the flies with the Y^L (the not-yellow flies) it is evident that penetrance of pod is increased in both the males and the females. In the latter, the increase in penetrance is greater, and thus the incidence of pod in males and females is brought to about the same value. This is good evidence for the assumption that the Y^L chromosome completely replaces the effect of the whole Y chromosome in the pod-K females, and that the additional heterochromatin also enhances penetrance of pod in the males.

E. PODOPTERA M(3)124

In the preparation of the combination of this stock with yellow, a marked decrease of penetrance of pod again resulted. The sex ratio of podoptera in pod-M(3)124 is always 1 : 1 (see table 11). For both males and females the controls are considerably lower than the Y^L group (table 122). The addition of extra heterochromatin thus results in increased penetrance of podoptera in females as well as in males of pod-M(3)124.

TABLE 122
Pod-M(3)124 WITH EXTRA Y

Cross	yellow		not yellow		pod yellow		pod not yellow	
	♀	♂	♀	♂	♀	♂	♀	♂
(yy Y^L × pod y) × pod y. .	327	273	207	209	5	3	13	11
Penetrance of pod					1 5	1 1	5 9	5 0

5. SUMMARY OF PART III

1. The genetic analysis of the podoptera effect presents unusual difficulties not encountered in standard *Drosophila* work.

2. Information on the broad genetic basis of the effect is derived from outcrosses and subsequent selection.

3. In outcrosses with tetraltera the presence of a sex-linked factor is revealed both in reciprocal F_1 and F_2 . The very low penetrance in F_2 outcrosses is based on multiple factors of individually very low action, with geometric increase of the effect in combination, i.e., a situation which combines features of multiple and multiplicate factors.

4. By selection of tet phenotypes, homozygous tet flies with the original penetrance were selected as early as F_6 . Other percentages of penetrance selected agree with an assumption of two to three autosomal factors and one sex-linked factor, of individually small but multiplicative action. From the details, the action of the different factors may be estimated. The autosomal factors have a rather low effect, and the addition of the first chromosome factor doubles it approximately. The values derived and tabulated (table 59) for the individual and the combined effects of the tet factors are in agreement with the conclusions derived from selection experiments.

5. The low penetrance of podoptera G is mostly due to a single autosomal recessive. Selection and outcrossing experiments do not give further information.

6. Outcrosses in the pod-H line do not give much information about the pod factors. In F_1 with pod-H as mother, dominance of podoptera is observed. It will be shown below that maternal inheritance is involved.

7. Results of outcrosses of podoptera K indicate again the presence of two to three autosomal pod factors. The specific feature here is that females show

the pod phenotype only in the presence of an extra Y chromosome in addition to the homozygous pod factors.

8. In podoptera M(3)124, two to three autosomal and one weak sex-linked factor seem to produce the effect. The Minute mutant interacts with these factors and plays a major role in increasing the penetrance controlled by the other factors.

9. In tet a powerful sex-linked tet factor was found. By crossing tet females with foreign attached X chromosomes to tet males with the X chromosome from the tet stock, it is possible to separate the effects of the autosomal tet factors from those of their combination with the sex-linked factor. The results agree roughly with those summarized in paragraph 4. But the extra Y in the females has a strong inhibiting effect upon the penetrance of tet.

10. The special X chromosome of the tet-100 stock (100 per cent penetrance) when acting alone is six times as powerful as that of the selected tet stock and correspondingly powerful in recombination with the autosomal factors.

11. The tet-100 stock originated from a cross of tet with M(3)124. The facts prove that a sex-linked factor from the Minute stock was inserted by crossing over into the tet X chromosome. The powerful action of the tet-100 X chromosome is thus due to the presence of the two tet (pod) factors.

12. An exact localization of tet and pod factors is extremely difficult, because of the low penetrance in outcrosses and because the marker stocks themselves introduce pod factors or act as enhancers or pseudoalleles of pod factors.

13. Standard experiments with tet show the absence of a major tet factor in the fourth chromosome and the presence of tet factors in all others. The same is true for pod-G, except for the factor in the X chromosome.

14. The principal pod factor of pod-H is in the second chromosome, although a low incidence of podoptera is found even if the second chromosome is replaced. Therefore, one or more of the other chromosomes have pod factors that have small independent action and that collaborate with the second-chromosome factors.

15. In pod-K the major factor is also in the second chromosome.

16. In pod-M(3)124 there is again a pod factor in the second chromosome. The X chromosome contains only minor pod factors. But the most powerful pod agent is the Minute mutant in the third chromosome.

17. In view of the difficulties inherent in the presence of pod factors in the marker stocks and (or) pseudoallelic action of some factors in these stocks, a chromosomal analysis was performed by isolating individual chromosomes of the pod stocks by means of crosses with dominant marker stocks.

18. In pod-G the isolated second chromosomes have the highest action, the third have the next highest, and the X chromosomes the lowest. Combined, the X and third chromosomes are multiplicative, but the X and second combined fail to show the expected increase over the action of the second chromosome alone. Most unexpected is the failure of the isolated chromosomes to restore the original conditions of penetrance of podoptera when all the chromo-

some of the isolated lines are recombined. It is probable that the fourth chromosome contains a pod factor having a negligible effect alone but a strong action when in collaboration with the other factors. In addition, the heterozygous pod factors, although recessive, seem to collaborate with the homozygous ones in an enhancing capacity.

19. In tet also, the second chromosome has the strongest effect alone; X and third combined have a roughly additive effect, X, second, and X, second, third have an exponential effect; but second and third combined have a smaller effect than the second chromosome alone. In the females all effects are more irregular than in the males. Again, the high penetrance of the homozygous stock is not recovered by selection after outcrossing. The most probable explanation is that the fourth chromosome, which alone has no discoverable tet effect, acts strongly in collaboration with other chromosomes. A conclusive test was technically impossible.

20. In tet-100 the recombination of first isolated X, second, and third chromosomes restores the original penetrance. Tests support the role of the fourth chromosome just mentioned.

21. In pod-H the incidence of podoptera is dependent upon the number of pod-X chromosomes and autosomes present: 2, 3, X > 2, 3, X Y > 2, 3 > 2, 3 Y.

22. The Y chromosome of pod-H when combined with pod-X in the males enhances the expression of podoptera phenotypes, but may act as an inhibitor when combined with a nonpod-X. This inhibition is expressed more strongly when the pod-Y is in the mother's genome ($\underline{X}Y^p$) as well as in that of the offspring, than when it is in the genome of the offspring alone. The rather complicated details have been summarized on p. 211 and p. 218.

23. The pod factors in the other chromosomes of pod-H act as dominance enhancers of the second-chromosome pod factor when it is heterozygous.

24. The isolated chromosomes of pod-M(3)124 produce less than 1 per cent podoptera, except in the combination of the isolated second chromosome with the Y chromosome of pod-M(3)124 in both males and attached X females. Here a penetrance of 3.1 per cent is obtained. The apparent multiplicative effect may, however, be due to the presence of the marker chromosomes (Hairless and Stubble) rather than to an interaction of factors in the two chromosomes of the pod stock.

25. The lines with isolated chromosomes of pod-K gave very irregular results. The second chromosome produced high penetrance only in one of the three lines in which it was isolated. The third chromosome combination with the X chromosome produced a higher penetrance than the second chromosome. These results do not agree with expectations on the basis of F_2 and backcross data with marked stocks.

26. Exact localizations within the chromosomes are very difficult. They had to be performed with isolated chromosomes. The sex-linked factor of tet could be localized to the right of forked, i.e., probably in the heterochromatin. For tet-100 an approximate value of 7 and 8 units to the right of forked was found. The second chromosome tet factor was found to be about 8 units to the right

of light, but this value is not very reliable. The pod factor in the second chromosome of pod-K may be in the same general region. The third-chromosome factors with their low penetrance are most difficult to localize. For tet a location between 25 and 65 may be assumed. In pod-M(3)124 the third-chromosome pod factor must be identical or closely linked with the Minute (3)124 locus.

27. The phenotypes of pod and tet are similar in some respects and differ in others. The problem arises whether the pod and tet factors can replace each other as a whole or in part when combined in the same individual, e.g., could the powerful X factor of tet also collaborate with the second chromosome factor of pod? Another problem is the specificity of the different pod factors in regard to their typical phenotype which segregates out of crosses, where traceable. The tests were made by crossing the different stocks and by combining isolated chromosomes from the different pod stocks.

28. These experiments showed that there is no allelism of the pod factors in the different stocks, and that they cannot simply replace each other in combinations. On the other hand, there is a certain amount of positive effect of interaction, the details of which point to what might be termed pseudoallelism between the different factors. The clearest results were obtained for tet and pod-G where the factors of one stock cannot collaborate with those of the others, as proved for chromosomes X, 2 and 3.

29. No allelism or interaction was found between the pod factors of pod-G and pod-K or those of pod-G and pod-M(3)124. But there are indications that some autosomal factors are common to pod-M(3)124 and pod-K.

30. The presence of an extra Y chromosome in the females of pod-G and tet inhibits the penetrance of the autosomal pod effect.

31. The presence of the long arm of the Y (Y^L) in addition to the normal Y in pod males greatly enhances their penetrance. The details of these experiments suggest that the much lower penetrance observed in pod-G males as compared to pod-G females is not based upon an inhibiting action of the male Y.

32. Both in pod-G and tet the presence of an extra Y enhances penetrance in the presence of the sex-linked factors.

33. In pod-K the Y^L replaces the effect of the entire Y chromosome in the females, i.e., females with Y^L will show a penetrance of pod similar to that of the males. In the males the Y^L increases the penetrance of pod.

34. The Y^L increases penetrance of pod in both the males and the females of pod-M(3)124.

PART IV. DOMINANCE, PSEUDODOMINANCE, RELATION OF PODOPTERA TO DOMINANT MUTANTS

In all the lines studied by us the pod and tet factors acted as recessives (with the apparent (not real) exception of pod-H; see Part V). When isolated chromosomes of these lines were tested for dominance of the pod factors in them, the results were again negative. But when the pod lines were crossed to stocks with dominant markers, the pod factors frequently behaved as partial dominants. When this phenomenon was studied in detail, remarkable relations between pod and the dominant, homozygous, lethal third-chromosome mutant Beaded (Bd, 3-93.8) were found. This led to a further analysis which revealed that the incompletely dominant mutant Beadex (Bx, 1-59.4) also produced partial dominance of the pod factors. Since there appeared to be some resemblance between the genetic action of the Minute mutants and the pod factors, the genetic relationship between a number of Minutes and pod was studied. All these data constitute an interesting group of facts of considerable importance for the whole problem.

1. DOMINANCE

In table 123 a number of crosses of pod lines with wild-type stocks and recessive marker stocks are assembled to show that, with the exception of pod-H, which is a special case (see below), pod and tet are recessive. Some apparent exceptions which were found when the experiments were extended are also shown in the table. In no. 15 one pod female was found among almost 3,000 flies. It is known from many tests that our isogenic Canton stock contains podoptera factors, and an occasional pod fly is also found in the ordinary Canton stock. In one of the matings of this group (no. 15) the Canton male used must have contained a pod factor. The same explanation may be used for the results of no. 31. The rucuca stock is another stock which contains occasional pod flies; hence the results of nos. 21 and 22. (It should be added here that all the tet crosses of this table were made with tet flies not containing the sex-linked tet factor.) Bran is a mutant at the arc locus derived originally from the same stock from which pod-G was obtained later (see Goldschmidt, 1945). In the crosses 23-28, the pod factors in the bran stock behave as if they were allelic to the tet factors. This may also be assumed for the attached X stock used in cross no. 30. It was shown in an earlier section that this kind of allélism—spurious allélism, or pseudoallélism—is typical for all pod factors as far as can be ascertained.

The terms "spurious allélism" and "pseudoallélism" have been used before in genetical literature, although no exact definition has been attached to them. In recent papers, Lewis (1945) used the term "pseudoallélism" for an allelic interaction between different but adjacent loci with nearly identical phenotypic effects—loci which he considers to have originated as repeats. In order not to create a new term, we shall use the same term also for an allelic action in which the loci are not adjacent and may be located even in different chromosomes. In a general way, interactions of the type studied here may be

of different kinds. When we speak of an enhancer (we are dealing with dominants in this chapter) we mean that the action of a homozygous recessive is increased in the presence of a certain dominant. If the recessive type becomes

TABLE 123
OUTCROSSES OF pod AND tet (EXCEPT pod-H)
(tet + = normal phenotype in tet stock; I-V = classes of expression)

No.	Mother	Father	F ₁		
			+	tet or pod ♀	tet or pod ♂
1	Ore wild.....	tet sel. V.....	766	..	..
2	Ore wild..	tet sel. I.....	240	..	..
3	Ore wild.....	tet sel. III.	396	..	..
4	Ore wild..	tet sel. +.. ..	166	..	..
5	Ore wild..	tet sel. II.....	516	..	..
6	Florida.....	tet sel. I.....	472	..	..
7	Canton.....	tet sel. +.. ..	960	..	..
8	al dp b lt px.....	tet sel. +.. ..	448	..	..
9	st sr e ^a ro ca.....	tet sel. +.. ..	36	..	..
10	se ss k e ^a ro.....	tet sel. +.....	17	..	..
11	ss ^a	tet sel. +.....	268	..	..
12	rucuca.....	tet sel. +.....	164	..	..
13	dp pr px.....	tet sel. +.....	120	..	..
14	al dp b lt px/Pm dp b.....	pod iso.....	50	..	..
15	pod-G iso.....	Canton.....	2,687	1	..
16	pod-G iso.....	al dp d b c px sp/Cy sp.....	250	..	..
17	pod-G iso.....	st sr e ^a ro ca.....	223	..	..
18	pod-G iso.....	se ss k e ^a ro.....	209	..	..
19	pod-G iso.....	ss ^a	251	..	..
20	pod-G sel. III.....	bw;e;ey.....	343	..	..
21	pod-G iso.....	rucuca.....	314	..	1
22	tet +.....	rucuca.....	164	..	1
23	bran.....	tet +.....	153	1	..
24	bran.....	tet +.....	159	..	..
25	bran.....	tet +.....	140	..	1
26	bran.....	tet +.....	105	..	2
27	bran.....	tet +.....	112	..	..
28	bran.....	tet +.....	150	..	..
29	pod M(3)124.....	Canton.....	96	..	..
30	<u>Y</u>	pod-M(3)124.....	680	2	2
31	pod-K.....	Canton.....	759	..	4
32	<u>Y</u>	pod-K.....	607	..	1
33	Canton.....	pod-K.....	126	..	..

more or less visible in the heterozygous condition in the presence of another dominant (or homozygous recessive) we call this a dominance modifier, and if the modifier has no other known action than the enhancing of dominance, we call it a dominigene. A situation is also imaginable in which a dominant acts as well upon the heterozygote as upon the homozygote. In this case the modifier is both an enhancer and a dominance modifier. Another type of

modifier or enhancer is one which increases (or decreases) the penetrance of a heterozygous dominant. We shall have to mention soon the increase of penetrance of *Bd/+* in the presence of certain inversions or *Minutes*. This interrelation cannot properly be called dominance modification—it deserves a special term.

How is pseudoallelism to be distinguished from the modifications mentioned? The decisive point is that two completely recessive loci with the same effect show a positive visible effect (e.g., *podoptera*) when both are simultaneously present as heterozygotes. In the cases already mentioned the effect was lower than that of either homozygote. One could object that we are dealing here with multiple factors, as shown above, and that therefore two single factors might have an additive combined action which lifts the effect above the threshold of visible effect. If this is the case one should expect heterozygotes of more powerful factors, e.g., those in the second-chromosome or the X-chromosome tet factor, to show some dominance. But they do not.

If, now, we cross the *podoptera* factors in isolated chromosomes with lines with dominant factors and find a *podoptera* effect in the heterozygote, the possibilities are as follows: (1) The stocks in question contain alleles of the *pod* factor used, which do not become visible in the balanced marker stocks. This explanation is difficult to disprove. But it is rather improbable that the effect would be found generally and independent of the marked chromosomes, whereas it is absent in crosses with recessive marker stocks. (2) The dominants themselves act as dominance modifiers. (3) The stocks in question contain low-penetrance *podoptera* factors which show pseudoallelism with those in the *podoptera* stocks. It will be shown that pseudoallelism is involved.

Crosses of *pod* and *tet* with stocks containing dominant markers show a remarkable result. Almost all such crosses contain *pod* flies. (Nos. 9, 10, and 12 in table 124 are the only exceptions; the *tet* males in no. 9 are produced by the sex-linked *tet* factor.)

Tests of the dominant marker stocks show invariably that they contain *pod* flies, some fewer, some more. As wild-type and recessive marker stocks may or may not contain *pod* factors, there must be some reason for the ubiquity of those factors in dominant stocks. If crosses of these stocks to *pod* lines produce a significant penetrance of *pod* and in outcrosses to wild type no *pod* is found, thus excluding dominant *pod* factors, the dominant stock may contain weak alleles of *pod* and it may enhance the compound, or the dominant simply enhances the heterozygous *pod* factors, or the dominant itself acts as a pseudoallele to *pod* factors or is linked with a recessive pseudoallelic *pod* factor. It is also possible that more than one of these agents are working simultaneously. In table 124 only a few examples are recorded (nos. 1–8 were checked simultaneously under identical conditions). This study was not amplified, when it was found that the dominants *Beaded*, *Beadex*, and the *Minutes* are best suited for further study, but in view of the presence of *pod* effects in the dominant stocks themselves the explanation by pseudoallelic action of recessives in the stocks or pseudoallelic action of the dominants themselves is preferred.

2. Beaded AND podoptera

The dominant mutant Beaded was given attention when it was found that scalloping of the wings was one of the pleiotropic actions of pod (see description above, p. 98). The scalloped flies were tested for an independent mutant, and this testing included a check for Beaded. Beaded is homozygous lethal, only slightly dominant in the heterozygote $Bd/+$, but is 100 per cent dominant in the presence of certain inversions (see Goldschmidt and Gardner, 1942). Thus, a mutation to Bd can be recognized at once by making a compound with a suitable inversion.

TABLE 124
Pod AND DOMINANTS

No.	Mother	Father	F ₁			Remarks
			+	pod ♀	pod ♂	
1	pod-G I-III.....	D Sb/In.....	298	2	2	pod are D Sb and In
2	pod-G I-III.....	S/Cy.....	170	1	..	pod are S
3	y Cy/Pm S/H.....	pod-G iso.....	405	4	1	pod are 4 Sb, 1H
4	CIB Cy/Pm S/H.....	pod-G iso.....	400	1	..	pod are 1 Sb
5	pod-G I-III.....	H/Xa.....	293	1	..	pod are H
6	pod-G I-III.....	Ly/D.....	601	1	1	pod are Ly
7	pod-G I-III.....	R/Mé.....	317	..	1	pod are Mé
8	pod-G I-III.....	e bx ^D /In.....	508	1	1	pod are bx ^D
9	tet.....	Cy/Pm H/Sb.....	1,139	..	32	
10	pod-K.....	Cy/Pm H/Sb.....	300	..	..	
11	Cy/Pm H/Sb.....	pod-K.....	158	1	..	
12	Cy/Pm H/Sb.....	pod-M(3)124.....	496	..	..	
13	pod-M(3)124.....	Cy/Pm H/Sb.....	236	1	..	

An inbred pod-G line which rarely contained scalloped flies was used to study the inheritance of scalloping. The pedigree, table 125, shows the incidence of this type. The same irregularities in regard to scalloping as described above (p. 98) are found. The scalloped male from 9989 was mated to a standard $Bd/In(3R)C, 1(3)a$ stock. ("Standard Bd " always means the original Columbia balanced Bd stock.) The results of the matings are summarized in table 126.

This table shows that the combination of scalloped pod with Bd produces 34.8 per cent Bd , including some high-grade Bd flies. In a mating of not-scalloped pod with Bd , only 6 per cent Bd of low and medium grade were found. (100 per cent Bd is produced by the compound of Bd with the Payne inversion.) But the same Bd heterozygous for a Canton third chromosome had only about 1 per cent penetrance. If the scalloped flies from pod contained a heterozygous Bd allele such as Bd^{pod} , the cross would have been $Bd/In \times Bd^{pod}/+$. Bd homozygous is lethal, and if Bd^{pod}/In also has 100 per cent penetrance, as proved by the high-grade expression, a penetrance of 33.7 per cent (0.4 per cent penetrance of Bd in the group $Bd/+$ has to be added) is to be expected, and approximately this was found. The F₁ Bd flies are therefore

either $Bd/+$ or, in the majority of cases, Bd^{pod}/In . As both genotypes may also appear as normal phenotypes (99 per cent of $Bd/+$, a few of Bd^{pod}/In), and as normal F_1 flies may also be $In/+$, different combinations may result from breeding $F_1+ \times +$. The one cross recorded (no. 10801) was clearly $Bd/+ \times In/+$. The expectation in this case is a little over 25 per cent Bd . F_2 from Bd phenotypes may also be genotypically different. Unfortunately, only a single mating was made with a not-virgin female, so that the resulting penetrance of approximately 50 per cent Bd is probably a mixture of the

TABLE 125
ORIGIN OF scalloped MALE 9989 IN $pod-G$

Generation	No.	Parents	Normals	pod		scalloped
				♀	♂	
F_1	9505	9309 ²	189	2	4	...
	9506	9309 ²	270	2	..	1 ♀
	9507	9309 ²	244	..	..	...
	9508	9309 ²	174	1	1	...
	9509	9309 ²	264	..	..	...
F_2	10000	9506 ² ♀ scalloped.....	30	..	..	...
	9999	9505 ²	213	..	..	...
	10042	9505 ²	220	..	1	...
	10043	9505 ²	226	1	..	...
	9988	9505 ²	18	..	..	...
	9989	9505 ²	112	1	1	1 ♂
	9990	9505 ²	86	..	1	1 ♂
F_3	10275	10000 ²	252	1	4	...

matings $Bd^{pod}/In \times Bd^{pod}/In$ and $Bd^{pod} \times Bd/+$. Here, however, some flies were found with straplike wings, and they reproduced their kind (with a certain amount of variation) with 100 per cent penetrance. Thus, a line Bd^{pod}/In had been selected, and therefore the scalloped pod fly must have contained this higher Bd allele. A test of the salivary gland chromosomes (by A. H.) actually revealed the presence of the inversion (3R)C from the Bd stock in the new Bd line.

If we return now to the controls in the table below, we find that the phenotypically high grade Bd F_1 flies, i.e. Bd^{pod}/In when bred to isogenic pod produced 38.5 per cent penetrance of Bd and a very high grade of expressivity, whereas the same isogenic pod stock crossed with standard Bd/In produced only 6 per cent penetrance of a medium grade (which itself is 5 times the penetrance of $Bd/+$). The latter result excludes the normal presence of Bd^{pod} in the isogenic pod stock. The compound of pod factors with third-chromosome inversions known to act as enhancers of Bd (details of this enhancing effect in Goldschmidt and Gardner, 1942) never produced either a Bd or a pod phenotype. On the other hand, the penetrance of Bd was enhanced in the

heterozygote with pod as compared with Bd/Canton. Furthermore, the control cross $Bd^{pod}/In \times pod$ with 38 per cent penetrance and high expressivity requires the presence of a Bd allele in the isogenic pod male used (which certainly did not contain an inversion).

How can all these results be explained? A probable explanation may be derived from the following facts: 1. A number of nicked individuals in the pod stock had been tested previously for allelism with Bd with negative results. 2. The fly used initially in the experiment just described showed more scalloping than usual. 3. In the isogenic podoptera line there was a second

TABLE 126
OFFSPRING OF pod scalloped

Generation	No.	Cross	Percentage Bd	Grade	pod
F ₁	10580	Bd/In $\times$ pod scalloped 9989.	34.8	medium-high	5 Bd
F ₂	10801	10580 ² + $\times$ + ...	33	low-medium	
	10802	Bd $\times$ Bd (virgin?)	50	some strap	
F ₃	11009	18801 ² + $\times$ Bd	17	low-medium	
	11011	18802 ² strap	100	extreme (strap)	
	11012	18802 ² strap $\times$ + ...	30	low-medium	
Controls		Bd/In $\times$ Canton.	1.4 ^a	low	3 Bd
		10580 Bd $\times$ Canton.	8.4 ^a	low	
		Bd/In $\times$ Bd/In.	100	low-high	
		10580 Bd $\times$ Bd/In	33.3	low-high	
		10580 Bd $\times$ pod iso.	38.5	med.-very high	
		Bd/In $\times$ pod iso.	6.0 ^a	low-medium	

* Actual penetrance of heterozygous Bd; otherwise, percentage in brood.

instance of the appearance of a powerful Bd factor (see below). One possible interpretation which, however, is not considered to be the correct one, is that two different things are involved here: 1. The podoptera G stock contains a third-chromosome factor which is a low allele of Bd, viable in homozygous condition as well as in the compound with higher Bd alleles. This allele has a pod effect of low penetrance and simultaneously a Bd effect (homozygous) of still lower penetrance. But the compound Bd/bd^{pod} has five times the penetrance of $Bd/+$. 2. bd^{pod} has a tendency to mutate into Bd^{pod} which does not increase the penetrance of its pod effect but causes it to act as a higher allele of Bd, as described. A fact which is in favor of this interpretation is that the podoptera flies in F₂ (table 126, no. 10801) were all simultaneously Bd. It should be pointed out that if this interpretation is correct, we are dealing with a new example of the thus far unique spineless-aristopedia situation (see Bridges and Brehme, 1944), again involving a homoeotic effect (of course with the difference caused by the specific features of each mutant). But there is also the possibility of another explanation, namely, that pod shows pseudo-allelism to Bd and to Bd^{pod} , and that therefore a heterozygous combination (compound) of pod with either acts as if a low Bd allele had been introduced

by the podoptera stock. The data to be presented will bear out this alternative.

We just mentioned the reappearance of a possible mutation of bd^{pod} to Bd^{pod} in one isogenic pod line (different from the selected homozygous pod line of the case just discussed). The details are as follows: In an isogenic pod line (brother-sister single-pair matings) 2 scalloped flies appeared in the third generation:

F₁ 9926 294+, 2 ♀ scall., 1 ♀ 2 ♂ pod

One of the scalloped flies was bred to a normal brother:

$$F_2 \quad 10286 = 9926^2 \text{ } \varnothing \text{ scall. } 137+$$

F₃ 10505 = 10286² 201+, 2 ♀ 2 ♂ pod

The other scalloped female was tested with Bd/In(3R)C:

F₁ 10324 = 9926 ♀ scall. × Bd/In 134+, 1 ♀ 2♂ pod

F₂ 10526 = 10324² + × + 229 +, 1 ♀ 2 ♂ pod

10543 = 10324 ²	+ × +	all +
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$$10540 = 10324^2 \quad \text{Bd} \times + \quad 131+, 68 \text{ Bd low-high}$$

10541 = 10324² + $\times$ Bd 93+, 90 Bd medium—very high

10527 = 10324³ Bd × Bd 33 per cent Bd up to very high, 1 ♀ 1 ♂ pod

$$10542 = 10324^2 \quad \text{Bd} \times \text{Bd} \quad 76+, 93 \text{ Bd}$$
$$10543 = 10324^2 \quad \text{Bd} \times \text{Bd} \quad 34+, 69 \text{ Bd}$$

If the scalloped female was Bd^{pod}/bd^{pod} the expectations for the offspring are (see above): (1) a very small percentage of Bd in inbreeding; (2) in F_1 with Bd/In , about 33 per cent Bd ; (3) F_2 from normal $\times$ normal, either all normal, about 4 per cent Bd , or about 27 per cent Bd , depending on the genotype of the normal parents used; (4) F_2 from $Bd \times$ normal phenotypes, about 33 per cent Bd ; (5) F_2 from Bd , either all Bd , or, if one parent was Bd/bd^{pod} (6 per cent), about one-third Bd . Not all F_2 agree with this expectation. But in F_2 , strap flies from 10541 were extracted which must have been Bd^{pod}/In as before. They bred true to the extreme type exactly as in the case reported above. The cytological check (by A. H.) showed again the presence of the (3R)C inversion. Thus, the explanation of this body of facts is considered to be correct, as far as the appearance of a high Bd allele in podoptera is concerned.

This repeated appearance of a high Bd allele in the pod-G stock, as well as the fact observed at this occasion that Bd together with heterozygous pod has a higher penetrance than Bd/+, led to a detailed study of Bd in relation to pod. As pod, furthermore, had shown definite interaction with other dominants, the relation of Bd to other dominants was included in the study. The data obtained made it desirable to use also the dominant mutant Beadex (Bx) in a parallel study, and finally the Minutes were studied in their interaction with pod and with some of the dominants.

The first problem is that of interaction between Bd and the pod lines. Table 127 contains the F₁ data. Nos. 13 and 14 are the controls. No. 13 is the most reliable one, because the Florida line used in the cross is known to be free of pod factors, whereas other wild-type stocks are not. The penetrance (per cent dominance) of Bd is calculated for the entire brood. Actually, one-

TABLE 12

F₁ Bd × pod (o

Cross	+		Bd		pod or tet +		pod or tet Bd		Percentage pen. of Bd ^a		Total pen. of pod or tet		
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	+
											Bd		
1. Bd/In × tet 100.....	195	148	29	51	1	..	..	..	12.9	25.6	0.8	...	...
2. recipr. 1.....	116	80	25	32	2	25	..	7	17.5	27.1	...	4.8	1.4
3. Bd/In × tet.....	323	292	31	44	..	..	..	..	8.7	13.1	...	...	...
4. recipr. 3.....	241	179	40	92	2	14	..	6	14.1	33.7	...	2.0	0.7
5. Bd/In (3R)/C × pod 124/In {	120	128	26	38	..	..	..	..	17.9	24.1	...	...	...
(3R)/C.....	6M	10M	21M	31M	..	..	..	..	17.8	175.6	...	...	...
6. Bd/In × pod-H.....	216	223	44	70	..	..	..	..	17	23.9	...	...	...
7. Bd/In × pod-G.....	147	109	66	77	1	2	..	1	30.8	40.7	...	0.5	1.1
8. Bd/In × pod K.....	165	134	11	23	..	..	..	..	6.3	14.7	...	...	...
9. Bd/In × pod-G(2) Sb/H.....	216	200	26	49	..	..	..	..	10.7	19.7	...	...	...
10. Bd/In × pod-G(3) Cy/Pm.....	214	192	13	40	..	..	..	..	5.7	17.2	...	...	...
11. Bd/In × tet (2) Sb/H.....	348	285	8	15	..	..	..	..	2.3	5.0	...	...	...
12. Bd/In × tet (3) Cy/Pm.....	231	252	8	17	..	..	..	..	3.3	6.3	...	...	...
13. Control Bd/In × Fla.....	150	146	3	9	..	..	..	..	2.0	5.8	...	...	...
14. Control Bd/In × Ore/In.....	376	280	136	146	2	2	1	3	26.6	34.5	0.4	4.0	0.6

^a Penetrance of Bd is calculated for the entire brood, i.e., it has to be doubled for the heterozygote Bd/+ alone. If the father contained an inversion with the lethal of the Bd balancer, F₁ would contain one-third Bd/In, i.e., phenotype Bd, and one-third Bd compound with pod, which in no. 5 is Bd/M(3)124. If the compound of the two inversions is viable, as in no. 14, 25 per cent Bd phenotypes plus a few per cent Bd phenotypes in the Bd/+ group are expected.

half of the flies are Bd/pod and the other half In/pod. Thus, the penetrance values should be doubled in order to apply only to the Bd/pod combination. It should also be added that the expressivity of Bd in the heterozygote is strictly proportional to the degree of penetrance, i.e., with low penetrance Bd is expressed as small nicks or dents; the scalloping increases with penetrance, and in the case of 100 per cent penetrance of all Bd heterozygotes, high-grade scalloping and beading of the anterior edge of the wing are present. Thus, the control no. 13 shows 2 per cent penetrance (= 4 per cent for Bd/+) in the females and 5.8 per cent in the males. This is an unusually high penetrance for this heterozygote, but it is still much lower than in the experiments. It is difficult to calculate penetrance for control no. 14. The Oregon stock is known to contain pod factors and also a third-chromosome inversion which enhances Bd to 100 per cent dominance. Thus, one-fourth of the offspring is Bd/In and therefore phenotypically Bd. There are a small number of pod flies, one-half of them Bd. As the combination Bd/Oregon without the inversion should have a very low penetrance of Bd, the penetrance values of Bd which are above 25 per cent in this cross show that the pod factors in the Oregon stock enhance the penetrance of Bd/+. (We had seen the same above where we found a fivefold penetrance of Bd with heterozygous pod-G.) The relatively high incidence of pod phenotypes in this cross shows again that Bd acts as an allele or pseudoallele of pod factors. Nos. 1-8 in the table show results of the combination of Bd with different pod lines. The line pod-M(3)124 (used in cross no. 5) is balanced by In(3R)C, e, l(3)e, and Bd is balanced by In(3R)C, l(3)a. The homozygous inversion (3R)C is not lethal, but l(3)a has a dominant semilethal maternal effect on females carrying M(3)w (Bridges and Brehme, 1944) and also on those with the allele M(3)124. The class M(3)124/In(3R)C, l(3)a is therefore greatly reduced in number. Thirty-two of the phenotypically Minute not-Bd flies were tested for Bd by outcrossing to a stock containing In(3R)C. No Bd was found. It may therefore be concluded that all the flies tested were genotypically In(3R)C/M(3)124 and that in the individuals of the genotype Bd/M(3)124 penetrance of Bd was 100 per cent. No podoptera flies were found. This lack of pod flies suggests that the pod factors of pod-M(3)124 and the Bd stock are not allelic or pseudoallelic. Both tet and pod-G (nos. 1-4, 7) are free of inversions but enhance the penetrance of Bd considerably, as the column "penetrance of Bd" shows (to be multiplied by 2, as explained in table 127). Pod-G has the strongest enhancing (or allelic or pseudoallelic) action—over 80 per cent penetrance of Bd is found in the males. But tet also produced a high penetrance of Bd. In all the matings, except no. 3, pod or tet flies are found. In matings 2 and 4 the large number of tet males is based upon the sex-linked factor, so that only the females can be used for comparison in those two crosses. In no. 1, both sexes may be used. The penetrance of pod is of the order of magnitude found in the isogenic pod-G stock, but autosomal tet action is decreased below the penetrance ordinarily found. Thus, both tet and pod-G show pseudoallelism to Bd. Pod-H and pod-K (nos. 6 and 8) enhance the penetrance of Bd, but no pod flies appeared in F₁ of these two crosses (see R.F.₂ results). In nos. 9-12 the isolated second and third

TABLE 128

BACKCROSSES (EXCEPT THE F₂ NO. 3) OF BEADED × POD WITH POD (TET)

Cross	♀ +	♂ +	♀ Bd	♂ Bd	♀ M Bd	♂ M Bd	♀ M Bd	♂ M Bd	pod +		pod M		pod Bd		pod BdM		pen all Bd	pen + Bd	pen M Bd	pen pod		pen pod among (M) Bd/pod
									♀	♂	♀	♂	♀	♂	♀	♂				♀	♂	
1. (Bd. × M(3)124) ♀ Bd × father.....	122	133 ^a	24	45	26	37	7	6	6	1	..	..	10	6	33.4	15.6	17.8	11.8	5.7	44.4	..	
2. (Bd × M(3)124) ♀ + × father.....	54	52	..	..	..	..	..	..	8	6	..	..	..	..	..	..	..	13	10.4	..	..	
3. (Bd × M(3)124) ♀ ♂ M/Bd F ₁		2	..	..	40 (3+)	42 (2+)	..	..	..	..	..	..	4	4	..	..	..	8.7	8.0	..	..	
4. (Bd × pod-G) ♀ Bd × father.....	146	115	21	38	..	..	3	2	..	..	..	1	..	..	18.4	..	..	0.9	0.9	16.7	..	
5. (Bd × pod-H) ♀ Bd × pod-H.....	130	128	13	13	..	..	2	1	..	..	..	1	..	..	9.4	..	..	0.7	0.7	..	..	
6. (Bd × pod-H) ♀ + × pod-H.....	219	195	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	0.9	..	..	..	
7. (Bd × pod-K) ♀ Bd × pod-K.....	252	177	4	58	..	..	12	9	..	..	..	5	10	..	3.3 26.8	..	..	7.0	8.0	41.1	..	
8. (Bd × pod-K) ♀ + × pod-K.....	260	196	..	..	..	..	..	11	..	..	..	..	..	..	..	..	..	..	5.3	..	..	
9. (Bd × tet-100) ♀ Bd × tet-100.....	214	199	20	41	..	..	9	23	..	..	1	6	..	..	13.3	..	..	4.1	11.1	16.0	..	
10. (Bd × tet) ♀ + × tet (+).....	221 236	193 216	20	16	..	..	28 23	37 42	..	..	2	4	..	..	7.5	..	..	11.3 10.6	16.3 16.6	8.0	..	
1. (Bd × pod (2) ^b ♀ + × father (+).....	127 55	103 95	.. 38	.. 30	..	..	2 1	1	..	..	..	..	..	..	26.9	..	..	1.5	0.9	..	..	
2. Bd × pod (3) ^c ♀ Bd × father.....	138	116	2	2	..	..	..	..	..	..	..	..	..	1.5	..	..	..	..	..	..	..	
3. Bd × pod (3) ♀ + × father (Bd).....	46 47	46 53	1	4	..	..	..	..	..	..	..	..	..	5.1	..	..	..	..	..	..	..	
4. Bd × tet (2) ♀ Bd × father.....	153	115	..	4	..	..	..	..	..	..	..	..	..	1.5	..	..	..	..	..	..	..	
5. Bd × tet (2) ♀ + × father.....	95	95	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
6. Bd × tet (3) ♀ Bd × father.....	124	112	..	3	..	..	..	..	..	..	..	..	..	1.3	..	..	..	..	..	1.6	..	
7. (Bd × tet (3) ♀ + × father.....	106	101	..	..	..	..	..	1	..	..	..	..	..	..	57.0	..	..	..	..	7.2	40.0	
8. (tet-100 × Bd) ♀ Bd × father.....	35	23	45	41	..	..	..	3	..	..	..	2	..	..	..	..	..	..	..	..	..	

^a Includes M.^b pod (2) means the isolated second chromosome from pod.^c pod (3) means the isolated third chromosome from pod.

chromosomes of pod-G and tet are studied separately. Both chromosomes from pod-G enhance the penetrance of Bd considerably, whereas the isolated tet chromosomes do not do so. There are no pod flies, but since the homozygous isolated chromosomes produce only a very few pod (tet) flies, this is to be expected if Bd does not completely replace the action of the pod factors.

More information should be obtained if some or all of the pod factors are made homozygous by backcrossing (in Bd flies this is impossible for the third chromosome except by crossing over). But, as the second chromosome contains the most potent autosomal pod or tet factors, respectively, their homozygous combination with Bd/pod-3 can be studied in the backcrosses (Bd $\times$ pod) $\times$ pod. Table 128 contains these data. Where it was possible, the pod fathers were used for the backcrosses. The corresponding backcrosses with not-Bd flies, i.e., In/pod-3 $\times$ pod serve as controls; but in some cases the phenotypically normal F₁ females turned out to be Bd/pod. The most interesting result is that of nos. 7 and 8. In pod-K, females do not show the pod phenotype unless they contain an extra Y chromosome. Thus, in the backcross no. 8, no pod females appeared. But in no. 7 there were pod females and males both with and without Bd. The main pod factor in pod-K is in the second chromosome. Therefore, the backcross will result in (a) one-fourth 2-pod-K/+; Bd/3-pod-K; (b) one-fourth 2-pod-K/2-pod-K; Bd/3-pod-K; (c) one-fourth 2-pod-K/+, 3-pod-K/3-pod-K; and (d) one-fourth 2-pod-K/2-pod-K; +/+. Of these combinations, b and d are homozygous for the second chromosome of pod-K, and therefore a certain percentage of the males would be expected to be phenotypically pod. Actually, however, about an equal number of pod males and females were found. It therefore appears that the presence of Bd has the same influence on penetrance of pod in the females as does the Y chromosome. Although this assumption explains the pod females which are simultaneously Bd, it does not explain why there are also females in the pod not-Bd class. Penetrance of Bd is rather low, as the third column shows. Although it is enhanced by pod, as we know, it is certain that many, if not all, the pod + females are genetically Bd. It is remarkable that Bd has eight times more penetrance in males than in females in the not-pod flies. The explanation is this: In the stock pod-K, nicked wings appear as a pleiotropic effect of the pod factors. This nicking effect behaves like pod, i.e., it is not expressed in the females except in the presence of an extra Y chromosome. Since the nicks affect the wing tip only, they can be clearly distinguished from the scalloping of Bd. Therefore the 58 Bd males recorded were really Bd. This can only mean that the nicking effect of pod-K and the Bd effect are additive in the males but not in the females. Thus, the Y-chromosome-like effect of Bd upon the expression of pod in females does not extend to the nicking effect—a rather complicated situation from the point of view of development of these characters. The really important point, however, is that Bd acts like the extra Y chromosome.

The other crosses show that the penetrance of Bd is considerably enhanced by the presence of homozygous pod. In making a comparison with F₁ (table 127) we must take into consideration the fact that one-fourth of the RF₂ flies

have the genetic constitution of F_1 with Bd, another quarter, also with Bd, are in addition homozygous for the second chromosome of the pod stock. If Bd would enhance homozygous pod, much higher penetrance of pod should be obtained than in F_1 —which is not the case. Thus, the pseudoallelism of Bd and pod in F_1 does not mean an enhancing action of Bd; pseudoallelism seems to be the correct explanation.

We saw above that pod and tet crossed with different dominant marker stocks produced pod flies in F_1 , which might mean either that the dominant-containing stocks contained pod factors allelic to the factors of pod-G and tet, or that a pseudoallelism exists between pod factors of the pod stocks and those contained in many dominant stocks. Actually, two of the dominant marker stocks regularly had some pod flies. But in view of the negative allelism tests for the different pod lines it seems more probable that different, not allelic pod factors of different provenience exhibit pseudoallelism with each other, and that, in addition, the dominant mutants enhance the pod action (see discussion p. 251).

In view of the just discussed relations between pod and Bd, crosses were made between Bd and different dominant stocks. It should be kept in mind that F_1 between the dominants and Florida does not contain pod flies; further, that the penetrance of Bd is higher in the compounds with pod. Table 129 contains some F_1 combinations of Bd with second- and third-chromosome dominants. Penetrance of Bd is again calculated for the whole brood. In nos. 2-4, half of each dominant group does not contain Bd but contains the inversion instead, so that the actual penetrance of Bd applies to only one-half of the brood. Thus, the actual penetrance is twice the amount calculated (assuming equal viability of the flies with, and those without, Bd). All the dominants enhance the penetrance of Bd. Cy, D, and Mé are chromosomal rearrangements for which it is known (Goldschmidt and Gardner, 1942) that they enhance Bd. However, although S and Gl are not rearrangements, they act similarly. In no. 1 the second-chromosome dominant Bl is balanced over an inversion. One-quarter of the flies can thus be In/+; Bd/+. The high penetrance of this combination shows again the penetrance-enhancing action of the inversion, although it is located in a different chromosome. But Bl itself enhances penetrance of Bd to about the same level. In no. 5 the dominants are balanced over the same inversion as Bd. Therefore, one-third of the offspring should be Bd, one-third Bd/Dom, and one-third Dom/In. Actually, the Bd/In class is far too small, probably because their viability is lower. Sb H does not enhance penetrance of Bd but actually reduces it (only 0.5 per cent altogether). No. 6 is genetically comparable, as the two inversions are obviously lethal as a compound. The Bd penetrance is above normal but below Bd/D, obviously a compromise between the D and Sb actions working in different directions.

All these data show that homozygous podoptera factors enhance the penetrance of Bd, just as many dominants and chromatin rearrangements do. In addition, Bd shows an effect upon penetrance of podoptera in the pod-K stock by acting just as a Y chromosome in making expression of pod in the

TABLE 130

BEADEX, PODOPTERA AND DOMINANTS

Cross	♀ not Bx	♀ Bx	pod		pen. pod ♀	pen. Bx	Remarks
			♀	♂			
1. Control Bx.....	...	1,306	25	3	2.1 all	100	
2. Control Bx × Fla.....	438		3	2	0.7		
3. Control Bx × Canton.....	180	17		..		8 1	
4. Bx × y cv v ct f.....	127	28	2+	..	0.8	10.9	
5. Bx × Bd/In(3R)C,1(3)a.....	89	146	1 Bd	..	0.4	62.2	
6. Bx × Cy/Pm; H/Sb,In(3)Mo.....	90	18		..		16 7	Bx are 1 Cy Sb, 17 Pm Sb
7. Bx × Bl/In(2LR)/dp.....	2 +	75 +		..		97.4	
8. Bx × S/Cy E-S.....	85 Bl	3 Bl		..		3 4	
	56 Cy	1 Cy		..		1.8	
	57 S	1 S		..		1.8	
9. Bx × Sb H/In(3R)C.....	118 +	26 +	2 +	..	1.4	17.8	
	153 Sb H	0 Sb H	3 Sb H	..	1.9		Bx R missing 1 ♀ pod R, 1 ♀, 1 ♂ pod Mé
10. Bx × R/Mé.....	350	6	2	1	0.6	1.7	
11. Bx × D Sb/In(3)Payne.....	147 +	4 +		..		2.7	
	138 D Sb	3 D Sb		..		2.1	
12. Bx × D/Gl.....	62 D			..			
	54 Gl			..			
13. Bx × pod-G.....	457	110	20	9	3.4	18.7	1 pod is Bx
14. Bx × tet.....	166	118	1	5	0.4	41.4	All tet are +
15. Bx × pod-H.....	203	34	4	6	1.7	14.1	All pod are +
16. Bx × pod-M(3)124/In(3R)C, e,1(3)e.....	242	4 +	3	6	0.8	1.6	pod ♀ = 2 +, 1 M
	90 M	76 M		..	0.6	45.5	pod ♂ = 3 +, 3 M
17. Bx × pod-K.....	185	33		..		15.1	
18. Bx with extra YL.....	400 y	22 y	1	2	0.3	5.2	
	274 +	62 +	3	3	0.7	18.5	
19. F ₂ (Bx pod-G) ² pod × pod.....	50	55	2	1	1.9		pod are Bx
20. F ₂ (Bx × pod-G) ² pod × +.....	74	74	3	..	2.0		pod are Bx
21. F ₂ no. 1.....	...	669 ♀	40	9	♀ 5.7 ♂ 1.2		
	...	774 ♂		..			
22. F ₂ no. 2.....	62	62	3	..	2.4		2 ♀ pod +
23. No. 16 × father pod.....	29 +	0 +	4	4	6.4		Others M
	29 M	13 M		..	4.4	29.5	

females possible. On the other hand, pod was shown to have an effect pseudo-allelic to what had to be assumed to be very low penetrance pod factors in dominant marker stocks. The question is whether the reactions pod vs. Bd, Bd vs. dominants, and pod vs. supposed pod factors in dominant stocks are unrelated phenomena, or whether they represent all phenomena of pseudo-allelism of different types. To this should be added the strange fact that the rather rare Bd mutant appeared twice in pod lines. An answer to these questions appears premature.

3. Beadex AND podoptera

Our standard Bx stock (Bx 1-59.4) contained pod factors with a penetrance similar to pod-G in the females but lower in the males (see table 130, nos. 1 and 21). As it was known from earlier work (Goldschmidt and Gardner, 1942) that the dominance of Bx is enhanced by inversions, in a way similar to Bd, it could be expected that the relations between Bx and pod would be similar to those of Bd and pod. The relevant data are contained in table 130. There is first the problem of dominance of Bx. When crossed to the Florida stock which is known to be almost free of podoptera factors, no Bx flies were found among 441 F₁ females. (F₁ no. 2, F₂ no. 22). But in both F₁ and F₂ pod flies were present. This might mean that the very low pod factors of Fla show an action pseudoallelic with those contained in the Bx stock. The absence of pod flies in F₁ with stocks known to be free of pod factors, e.g. no. 7, precludes dominance. Only one other cross, no. 12, shows recessivity of Bx, and this is again a cross to a stock which is free of pod factors. But in the segregating, F₁ no. 9, Bx is completely recessive in the presence of Sb H, although pod flies are present. All other combinations show a varying penetrance of Bx. It is highest in the presence of inversions, most extreme in no. 7 with a second-chromosome inversion, lowest in no. 11 with a third-chromosome Payne-Inversion, similar in no. 16, and in between in no. 9. In no. 5 it is not possible to distinguish between the Bx-Bd interaction and that of Bx Inversion. The penetrance of 62 per cent leaves a minimum of 12 per cent to the action of the inversion. The Minute in M(3)124 (= pod-M(3)124) has an almost 50 per cent dominance-enhancing effect. It should be borne in mind that all Minutes enhance Bd to 100 per cent penetrance. The other pod lines (nos. 13, 15, 17) also have a considerable effect upon penetrance (dominance) of Bx. In the majority of crosses also pod flies are obtained. Since our Bx stock contains pod factors, the situation is the same as in the crosses of pod with dominants discussed above, and the interpretation should be the same, namely pseudoallelism with low-penetrance pod factors in the tester stock. Only in the case of Bx × pod-G (no. 13) is it suspected that the second-chromosome pod factors are really allelic (because of the high penetrance of podoptera in this F₁). Most of the dominants of this series which do not enhance Bx dominance, namely Bl, S, Cy, D, Gl, D Sb, do not seem to contain pseudoallelic pod factors. Also, pod-K did not show pseudoallelic pod action with Bx.

These results are similar to those obtained for Bd although not identical. The discussion of the results (p. 261) should also cover the situation for Bx.

Since Bd showed clear relations to heterochromatin (its effect can replace that of the extra Y chromosome in pod-K females), Bx was also tested (no. 17), and since F_1 did not contain pod flies, an extra Y chromosome, actually Y^L (see chapter on Y chromosome), was introduced into the Bx line. By using the same technique as described for tet (p. 240) the combination (no. 18) was obtained in which the yellow flies are the controls, and the not-yellow flies (+) contain the extra Y^L . The results show that the extra Y^L in Bx/+ greatly enhances dominance of Bx. Thus, Bx shows interaction with heterochromatin as pod does, and Bd actually can replace heterochromatin in one case.

TABLE 131
PENETRANCE OF PODOPTERA IN F_1 OF CROSSES OF pod-G
WITH MINUTE STOCKS

Minute	Total	Percentage of pod
M(3)B ³	3,656	0.2
M(3)w.....	2,387	0.2
M(3)36e (a).....	106	11.0
(b).....	2,356	0.3
M(3)be.....	1,941	0.3
M(3)S34.....	1,292	0.2
M(3)S39.....	1,150	0.2
M(2)B.....	1,283	...
M(2)S5.....	422	0.5
M(2)S11.....	673	0.5
M(2)S12.....	992	...
M(2)S13.....	393	0.2
M(1)n.....	764	0.3

4. Podoptera AND Minutes

The occurrence of small numbers of podoptera flies in many Minute stocks has been reported (p. 78). In addition to the study of the genetic interrelationship between pod-M(3)124 and other podoptera stocks, several different Minute stocks were crossed to pod-G flies in order to determine whether incidence of podoptera could be increased in any of the combinations. In table 131 the results of these crosses are shown. The percentage of podoptera obtained was even lower than that of the isogenic stocks or that of the inbred Minute stocks. There was, however, one exception. In the cross pod-G $\times$ M(3)36e, 13 podoptera flies were observed in a total F_1 of 119. When the cross was repeated on a larger scale no high penetrance of pod was found. More than half of the podoptera types obtained in F_1 were not Minute; their appearance must therefore be caused either by the enhancing action of the Minute balancers in combination with factors from pod-G, or by pseudoallelic action of heterozygous pod factors in F_1 .

Comparable crosses were made with the selected high-penetrance tetraltera strain. In table 132 the results obtained with different Minutes are presented. The reciprocal F_1 clearly show (nos. 1, 2) that a sex-linked tet factor is

present. A cytoplasmic factor is ruled out by the F_2 results. The one tet male in no. 1 was obviously caused in the same way as before (by pseudoallelism). One tet female was found in the cross no. 2, which again means that a low autosomal pod factor in the Minute stock is allelic or pseudoallelic to one of tet, if no dominant factors are present, e.g., if the Minute itself has no dominant pod action. (It should be remembered that the pod classes I-III cannot be distinguished from the tet classes, or at least, usually cannot.) Thus, we find for the sex-linked tet factor a penetrance of 11.4 per cent (in the attached X tests, 1.3 per cent). This shows that the sex-linked tet factor collaborates with the autosomal tet/Minute compound just as it does with homozygous tet autosomes. In the case of tet we found about 38 per cent penetrance for

TABLE 132
CROSSING SELECTED tet to THIRD-CHROMOSOME MINUTES

Cross	Broods	Numbers			tet and M
		+	♀ tet	♂ tet	
1. F_1 M $\times$ tet.....	5	503	..	1	tet ♂ is M
2. F_1 tet $\times$ M.....	3	263	1	17	tet ♀ is M

X-autosomal collaboration for part of the autosomal factors, and 60 per cent to 80 per cent for all. The present result, i.e., only 17 per cent in the compound, speaks more for pseudoallelic action than for allelism of tet and Minute factors.

5. SUMMARY OF PART FOUR

1. All the pod stocks studied here are completely recessive. The line pod-H is an apparent partial exception, for which the explanation will be given in the fifth part.

2. In a few crosses of pod with recessive marker stocks, pod flies appeared in F_1 in very low percentages. It could be shown that the marker stocks contained pod factors, and it is assumed that these are pseudoallelic to pod factors in the pod stocks. The meaning of pseudoallelism and other possible interpretations are discussed.

3. In the majority of crosses between podoptera and different stocks containing dominant markers a low percentage of pod flies is obtained in F_1 .

4. Most of these dominant-containing stocks have also a small percentage of pod flies. We assume that these stocks contain low-penetrance pod factors, pseudoallelic to those of the pod stocks, although other explanations cannot be excluded.

5. Scalloping of the wings is one of the pleiotropic effects of pod. Scalloped flies were frequently tested for allelism with Beaded (among others), and twice it was found that a high Bd allele, Bd^{pod} had arisen.

6. During the analysis it was observed that Bd, which has very low penetrance normally (it is homozygous lethal) increased in penetrance in the presence of heterozygous pod factors. This is true for all pod lines. Pod-

M(3)124 occupies a special position because it is a Minute and all Minutes increase penetrance of Bd to 100 per cent.

7. In the presence of homozygous pod factors the penetrance of Bd is not further increased. This points to the interpretation that the action of heterozygous pod upon Bd is not enhancing but rather is pseudoallelic.

8. In pod-K the females never show the pod character except in the presence of an extra Y chromosome. Pod-K females, however, show the pod phenotype in the presence of Bd. Beaded has here the same action as extra heterochromatin.

9. In view of the interrelation of pod and dominant marker stocks (points 3, 4) the effect of such dominants on the penetrance of Bd was studied, and the penetrance-enhancing effects of chromatin rearrangements in different chromosomes were restudied. Many dominants were found to have an enhancing action.

10. It is difficult to decide whether the reactions of pod with Bd, Bd with dominants, pod with dominants, the pod-K case, and the reappearance of the otherwise rare Bd mutants in pod stock are unrelated phenomena or not. No answer is given.

11. Beadex (1-59.4) was subjected to a similar study as Bd because it had been found earlier that some parallels existed (Goldschmidt and Gardner). Although Bx, if combined with pod and dominants and also with extra heterochromatin, does not interact exactly as Bd does, a considerable parallelism exists. (Our Bx stock is simultaneously a pod stock). Pod and also extra heterochromatin enhance Bx.

12. Many Minute stocks were found to contain podoptera. Compounds with pod show fewer pod flies than either one of the parent stocks, i.e., the pod factors are not allelic but are probably pseudoallelic. The Minute loci themselves do not act as enhancers or pseudoalleles.

13. In the presence of the X-chromosome tet factor, the tet/M compound collaborates (as does homozygous autosomal tet) to produce higher penetrance of tet. The data are more in favor of pseudoallelism between tet and factors contained in the Minute than of true allelism of factors in the two stocks.

PART V. SPECIFIC FEATURES OF PODOPTERA H

In addition to the specific podoptera characteristics of the pod-H strain which distinguishes it from the others, features characteristic only of this strain were found. They are maternal inheritance of the pod second-chromosome factor, or factors, and hereditary gynandromorphism, which is also determined by a second-chromosome factor maternally inherited.

1. MATERNAL INHERITANCE

Maternal inheritance (Toyama) is inheritance of a character of the egg before the meiotic division as recognized by the difference in F_1 flies of reciprocal crosses. F_2 behaves like an F_1 in ordinary inheritance, and F_3 like an ordinary F_2 . In crosses to normal stocks or to stocks with recessive markers, pod-H does not behave like a simple recessive, for reciprocal crosses show recessivity for pod if the mother comes from a normal stock, but dominance if the mother was pod-H. These facts suggested that maternal inheritance may be responsible for the difference in incidence of podoptera in reciprocal crosses, the apparent dominance of a pod factor, and extremely low incidence of podoptera in F_2 .

The further analysis was carried out by making use of the data in which reciprocal crosses had been made of pod-H with stocks containing markers in the X and second chromosomes (table 60), as well as other data pertinent to the question: e.g., reciprocal crosses with a stock containing markers in the third chromosome and reciprocal crosses of one of the pod-H replacement lines (X^H ; 2^H ; H/Sb) and a pod-G replacement line (X^G ; Cy/Pm; 3^G). These data are summarized in table 133. They include gynandromorphs because the expression of gynandromorphism is dependent upon homozygosity of the pod-2 chromosome. The other chromosomes do not have an enhancing effect, and probably have no independent "gynandromorph" factors.

If the principal pod factor is maternally inherited, penetrance of podoptera in F_1 pod $\times$ nonpod should be equal to that of the stock (2.5 per cent) and there should be no podoptera in the reciprocal cross. The F_2 should not have any podoptera, and the F_3 should have about 0.6 per cent penetrance. On the other hand, the F_1 result might be due to cytoplasmic inheritance. In this case the reciprocal F_2 , etc., should remain different, like F_1 .

In crosses of pod-H with recessive marker stocks the average incidence of podoptera with pod cytoplasm in F_1 is 1.88 per cent, nonpod cytoplasm 0.26 per cent; in F_2 : pod cytoplasm 0.18 per cent, nonpod cytoplasm 0.19 per cent; in F_3 : pod cytoplasm, 0.37 per cent, and nonpod cytoplasm, 0.43 per cent. In the case of the gynandromorphs in F_1 , pod cytoplasm had 2.37 per cent, nonpod cytoplasm 0.04 per cent; in F_2 : pod cytoplasm 0.02 per cent, nonpod cytoplasm 0.00 per cent; in F_3 : pod cytoplasm 0.07 per cent, nonpod cytoplasm 0.12 per cent. Although the data do not agree too well with the expected results for maternal inheritance, they do eliminate cytoplasmic inheritance and do show that maternal inheritance is in part responsible for the expression of the podoptera and gynandromorph phenotypes. The presence of the pod-Y

in the flies with the nonpod cytoplasm may in part contribute to the presence of the podoptera phenotype in F_1 and F_2 , because it has been shown in the replacement tests that both the X and Y have slight but independent pod effects, but when combined the incidence is relatively higher. However, as the difference between reciprocal crosses in F_2 is not significant, the difference may be due to chance.

The F_3 data show the greatest deviation from the expected results. Because of lack of significant difference between F_2 and F_3 in the incidence of podoptera and gynandromorphs, and the low incidence of both in the F_3 , comparisons were made of the incidence of podoptera and gynandromorphs in single-pair matings of F_2 flies. Of 50 such matings, 6 had both podoptera and gynandro-

TABLE 134
Pod-H: INCIDENCE OF PODOPTERA AND GYNANDROMORPHS AMONG PROGENY OF
SINGLE-PAIR MATINGS OF F_2 FLIES

Total bottles	Mother pod	Total flies	pod		gyn	
			Total	Percentage	Total	Percentage
6	1	1,058	13	1.23	9	0.85
19	2	4,610	41	0.89	..	
25	3	4,532	..		..	

morphs, 19 had only podoptera, and 25 had neither podoptera nor gynandromorphs (table 134). On the assumption that one-fourth of the F_2 females were homozygous for the pod-2 chromosome, one-fourth of the matings should have both gynandromorphs and podoptera. The number of matings without these two phenotypes is rather high. However, as the average number of flies per bottle was 180, it might be expected that, by chance, some of the matings would not contain the expected phenotypes.

The data from the reciprocal crosses of pod-H (replacement line) X^H ; 2^H ; H/Sb and pod-G (replacement line) X^G ; Cy/Pm; 3^G give further information concerning segregation of the pod factors and their relation to the maternal effect. The results from these crosses (table 135) for the first three generations are similar to those of pod-H $\times$ nonpod. The incidence of podoptera is higher in the F_1 and F_2 of the pod-H $\times$ pod-G crosses. Only in the F_3 of pod-G $\times$ pod-H crosses does the incidence of podoptera approach that of the reciprocal cross, and here gynandromorphs appear for the first time. Therefore it seems probable that in this case the production of gynandromorphs is dependent upon the maternal effect, and that the production of podoptera is in part dependent upon the maternal effect and the pseudoallelism between the pod-H and pod-G factors.

By use of these same data, because all chromosomes may be traced, the chromosome responsible for the maternal inheritance was determined. The results for F_3 , F_4 , and F_5 given in table 133 are rearranged in table 135 to show the effect of recombination of chromosomes upon the expression of gynandro-

TABLE 135

INCIDENCE OF GYNANDROMORPHS IN COMBINATIONS OF SECOND AND THIRD CHROMOSOMES

Chromosomes and cytoplasm	F ₃			F ₄			F ₅			Total		
	Total flies	gyn		Total flies	gyn		Total flies	gyn		Total flies	gyn	
		Total	Percentage		Total	Percentage		Total	Percentage		Total	Percentage
2H, 3G	183	7	3.83	989	3	0.30	382	..		1,554	10	0.64
	721	2	0.28	652	8	1.23	544	3	0.55	1,917	13	0.68
2H, H/Sb	122	1	0.82	667	3	0.45	397	3	0.76	1,186	7	0.58
	128	2	1.56	629	2	0.32	399	4	1.00	1,156	8	0.69
Cy/Pm, 3G	137	..		567	..		314	..		1,018	..	
	404	1	0.25	280	..		611	..		1,295	1	0.08
Cy/Pm, H/Sb pod-H Cyt..... pod-G Cyt.....	143	..		750	..		663	..		1,556	..	
	82	..		431	..		271	..		784	..	
Total pod-H Cyt..... pod-G Cyt.....	585	8	1.37	2,973	6	0.21	1,756	3	0.17	5,314	17	0.32
	1,335	5	0.37	1,992	10	0.50	1,825	7	0.32	5,152	22	0.43

morphs. If the pod-H second chromosome is present, the average incidence of gynandromorphs is 0.65 per cent, with neither the pod-G third chromosome nor the H/Sb chromosomes having enhancing effect. If the second chromosome is replaced, the incidence of gynandromorphs is less than 0.02 per cent. It seems probable that the factor responsible for the expression of maternal inheritance is in the pod-H second chromosome.

The effect of the X and Y chromosome was tested by rearranging the data for the pod-H second chromosome from table 135, in table 136 to show the penetrance of gynandromorphs, e.g., the maternal factor with pod-H and pod-G first chromosomes. Although the incidence of gynandromorphs varies in

TABLE 136
COMPARISON OF THE INCIDENCE OF GYNANDROMORPHS IN CROSSES HAVING
pod-2 AND pod-G OR pod-H X CHROMOSOMES

X chromosome	pod-H cytoplasm nonpod Y			pod-G cytoplasm pod-H Y		
	Total	gyn	Percentage	Total	gyn	Percentage
pod-G X ^{yw}	564	5	0.89	562	9	1.60
pod-H X or pod-II X/pod-G X ^{yw}	743	12	1.42	1,008	13	1.29
Total.....	1,307	17	1.60	1,570	22	1.40

the different combinations of X, the difference is in no case statistically significant. Thus, the X chromosome has no effect upon the expression of gynandromorphs. Further details of the genetic analysis of the gynandromorph "factor" will be presented below.

2. CHROMOSOME ELIMINATION

A. GYNANDROMORPHISM

When mosaicism occurs in a portion of the body which typically exhibits sexual characteristics, the individual is classified as a sex-mosaic or gynandromorph. Since the theory of origin of gynandromorphs by chromosome elimination was first formulated by Morgan (1914), a large body of evidence has accumulated which shows that in *Drosophila* chromosome elimination is the most frequent cause of gynandromorphism. The test for elimination of a whole chromosome is that the portion of the body having the remaining chromosome will exhibit the characters of recessive marker genes (if the normal chromosome is removed), the portion of the body typically exhibiting sexual characteristics will be male, and the predominantly male mosaics will be sterile (XO).

The following analysis was made with pod-H gynandromorphs: All male-looking gynandromorphs were mated first to females, then, if the cross proved to be sterile, to males. All matings proved to be sterile, i.e., the predominantly male gynandromorphs were unable to function either as males or females. On the other hand, 30 per cent of the gynandromorphs, predominantly fe-

male, produced offspring. These data would indicate that the malelike gynandromorphs were of XO constitution. This conclusion is substantiated by the fact that the gynandromorphs with male genitalia also had testes and male accessory reproductive structures.

To test for the type of gynandromorphism, pod-H flies were mated to flies having recessive markers over most of the length of the first chromosome (y w ec cv ct v f). Fifty-one gynandromorphs (2.09 per cent) were found among 2,241 flies. These were classified, first by the chromosome eliminated, and second by the region of the body affected (table 137). Three times as many

TABLE 137

ELIMINATION OF THE X CHROMOSOME IN pod-H GYNANDROMORPHS AND REGION AFFECTED

Gynandromorphs	Maternal pod H chromosome eliminated	Paternal marked chromosome eliminated	Mixture
Bilateral—complete.....	..	..	..
Bilateral—partial			
Head and thorax.....	1	2	..
Thorax and abdomen.....	..	1	2
Head, thorax and abdomen.....	..	1	..
Head.....	3	..	..
Thorax.....	6	3	..
Abdomen.....	..	23	1
Anteroposterior.....	..	1	..
No genitalia.....	..	5	..
Minute mosaic.....	..	2 (pod)	..
Total.....	10	38	3

paternal chromosomes as maternal ones were eliminated. In three cases both maternal and paternal chromosomes were eliminated, but in different regions of the body. It appears that either chromosome may be eliminated, but the pod-H chromosome is retained most frequently.

The second fact brought out by these data is that the elimination may not occur at the first embryonic division, because no bilaterals were observed. However, the partials had one characteristic in common; the mosaic region always expressed all the markers of that particular region. If the pod chromosome had been eliminated in the head region, the eyes were white and echinus, the bristles yellow and forked; if in the thorax, the bristles were yellow and forked and the wing (if it was included in the mosaic) was yellow, cut, and cross-veinless, and was also smaller. Therefore, it may be concluded that the sex mosaics in pod-H are the result of elimination of the whole chromosome rather than of somatic crossing over or one of the other known causes.

It would have been desirable to test the reciprocal crosses as well to determine whether the paternal chromosome is more frequently lost than the maternal, but as only two gynandromorphs were found among 1,888 flies, this could not be determined. However, as one fly showed loss of the maternal

chromosome and one loss of the paternal, it is probable that the effect is no more selective than in the first case.

It is generally accepted by most investigators that in *Drosophila* gynandromorphs are nonhereditary developmental anomalies. They occur spontaneously, with a frequency of about one to 2,200 normal flies (Morgan and Bridges, 1919). However, there is some evidence that gynandromorphism may be inherited, or at least may appear in higher frequency in certain strains (Sturtevant, 1929). Because of the high incidence of gynandromorphs in the

TABLE 138
THE EFFECT OF THE pod SECOND AND THIRD CHROMOSOMES UPON THE
PENETRANCE OF GYNANDROMORPHS IN pod-H

Lines	Total flies	Gynandro- morphs	Percentage gyn
2 repl; 3 repl.....	6,906	1(M)	0.01
2 repl; 3 pod.....	6,283	1	0.02
2 pod; 3 repl.....	6,902	103	1.49
2 pod; 3 pod.....	10,984	181	1.74

TABLE 139
THE EFFECT OF THE pod-X AND SECOND CHROMOSOMES UPON THE
PENETRANCE OF GYNANDROMORPHS IN pod-H

Lines	Total flies	Gynandro- morphs	Percentage gyn
X repl; 2 repl.....	4,732	..	
X pod; 2 repl... ..	8,457	2	0.02
X repl; 2 pod.....	6,123	71	1.16
X pod; 2 pod.....	11,763	213	1.81

pod-H lines (0.46 per cent in the pod-H inbred lines and 3.83 per cent in a high gynandromorph line), it seemed desirable to test, first, whether it was dependent upon a genetic factor, or whether its expression is independent of the genotype. The localization test was made by using the same method and the same data as for podoptera (table 80) but using the incidence of gynandromorphs in place of podoptera. Tables 138 and 139 summarize the data in the tests with different chromosomes present or replaced.

It is obvious from these data that the primary factor is in the second chromosome, the third has no effect, and the first acts only as an enhancer of the second-chromosome factor. Although pod-X, pod-2 has a higher incidence of gynandromorphs than repl-X, pod-2 (1.53 per cent to 1.16 per cent) the difference is not statistically significant ($\chi^2 = 2.66$). Gynandromorphs were rarely found in the pod-X, repl-2 line—two were found among 8,456 flies. As one of them was also Minute-like, the third-chromosome Minute-like factor (see above, p. 206) may also be responsible for a very low incidence of chromosome elimination, or both may be the results of accidental loss of the X chromosome during embryogenesis.

It was impossible to localize the "gynandromorph factor" to a specific locus in the second chromosome, because of the low incidence of gynandromorphs. However, indirect evidence suggests that it is identical with the pod second-chromosome factor, or so closely linked that crossing over is very rare. Among 50 single-pair matings of F_2 flies from crosses of pod to flies with first or second-chromosome recessive markers, 6 had a high incidence of podoptera and gynandromorphs, 19 had a low incidence of podoptera, and 25 had neither podoptera nor gynandromorphs (table 134). If the podoptera and gynandromorph factors had been independent, there would have been evidence of segregation in F_3 . Had they been in the same chromosome but many crossover units apart, gynandromorphs would have been found in bottles (single-pair matings) which had no podoptera, or in bottles with a low incidence of podoptera. Finally, in the crosses of pod-H with flies having recessive markers in the second chromosome, gynandromorphs and podoptera were recombinants with the same markers.

Although these facts are not conclusive evidence to substantiate the hypothesis that the gynandromorph factor and pod factor are identical, they do suggest that chromosome elimination in pod-H is an expression of the second-chromosome pod factor.

B. AUTOSOMAL MOSAICS

a. *Minute-like mosaics*

Mosaics other than sexual were observed several times. Flies with Minute-like bristles on one half of the thorax occurred infrequently. One fly (heterozygous for shaven) was shaven on the left side and had normal bristles on the right side. As both the Minute-mosaic and shaven-mosaic effects are characteristic expressions of haplo-IV mosaicism (Sturtevant, 1929), it may be concluded that the Minute-mosaic effect may be the result of elimination of the fourth-chromosome in some cases. The other cases are not so clear, but the "Minute factor" in the third chromosome is probably responsible for some of the mosaic bristle pattern effects as well as the pod action.

In the replacement lines the Minute-like flies and Minute-like podoptera flies were found only if the third chromosome was pod. Of 24,601 flies with the third chromosome present, fifteen (0.06 per cent) were Minute-like and podoptera, or Minute-mosaic-pod. The number of Minute-mosaic flies is very small, and the penetrance of the Minute-like factor is so low that decisive conclusions may not be drawn, but all facts point to the probability that the Minute-like factor in the third chromosome is responsible in a number of cases for the mosaic bristle pattern.

These data do not eliminate the possibility that the Minute-like and Minute-like mosaic flies are an expression of somatic mutation which occurred late enough in embryogenesis to affect only a part of the fly. But neither the Minute-like flies (which presumably would have the germ cells as well as the somatic cells affected) or the Minute-like mosaics (which may or may not have the germ line affected) produced any progeny with a high penetrance of

the Minute-like effect. Therefore, it seems probable that the Minute-like effect and in some cases at least, the Minute-mosaic effect is an expression of the low-penetrance Minute-like factor in the third chromosome.

It could also be argued that the permanently balanced lethal condition of the pod-H third chromosome (see p. 199) would prevent isolation of any new mutant. But it did not. Two dominant third-chromosome bristle mutants, each appearing first as a single individual, were extracted without any difficulty. If other mutants could be extracted, the Minutes should also be extractable if they were actually individual mutations.

The Minute-like factor could not be localized to a region of the chromosome. Among 3,382 F_2 flies after outcrossing to a stock having the recessive markers *st sr es ro ca* in the third chromosome, only two Minute-like flies were observed, and both were nonrecombinants with the markers.

b. The second- and third-chromosome mosaics

Because of the high frequency of chromosomal elimination in case of the X, and some evidence of a similar phenomenon for the fourth, the second and third chromosomes were tested for evidence of elimination of a part of the chromosome (the fly is not viable if the whole chromosome is eliminated) and somatic crossing over.

The test of the second or third chromosome was made by using the data from an earlier experiment in which the pod flies had been mated with flies having recessive markers covering most of the length of the second or third chromosome. Table 140 records the number of flies which had a phenotype like one or more of the mutants in the nonpod marked stock. As approximately equal numbers of females and males showed these effects, only the total numbers are recorded.

The largest group is the Minute-like mosaic group. There was no evidence of elimination of a part of the chromosome or somatic crossing over, because the Minute effect appeared as frequently with all the markers as without them.

The second largest group includes the flies that appear to be dachs, e.g., having all legs reduced. All the "dachs" flies were tested. Most of them were sterile (which is also true for standard dachs), but the ones which were fertile gave no dachs (nonmarked) flies in either the F_2 or the F_3 progeny. It might be postulated that the dachs effect was a somatic mutation expressing itself when in compound with dachs in the homologous chromosome or a deficiency for dachs. However, four of the seven "dachs" flies were found among flies which did not have a chromosome containing dachs.

The critical evidence that the "dachs" effect is not a somatic mutation or a deficiency but a part of the pod effect was shown in the analysis of pleiotropic effects. The legs of podoptera flies are frequently abnormal, and there is a high degree of correlation in the bilaterals having wing and leg effects. In the majority of cases, if the podoptera effect is restricted to one side, the abnormal legs are on the same side, but in a few cases the opposite side may be affected, or all the legs may be abnormal. In addition, a number of flies without the podoptera phenotype may have abnormal legs. In the crosses to recessive

TABLE 140
TESTS FOR SECOND OR THIRD CHROMOSOME ELIMINATION IN pod-H

Loci tested	Total flies	Effect like recessive marker					Minute-like		
		daoba	One wing dp	M mos. px-like	M mos. e	$\frac{1}{2}$ sv	M mos.	M mos. pod	M pod
II chromosome	F ₁	1	..	..	..	..	..	..	..
	F ₂	..	..	..	..	..	..	..	..
	F ₃	1	1	..	..	..	2	..	..
al dp d b c px sp	F ₁	1	1	1	..	..	3	..	..
	F ₁	4	..	..	..	..	..	..	..
	F ₂	..	..	..	..	..	..	..	..
III chromosome	F ₁	..	..	..	..	..	3	1	..
	F ₂	..	..	..	1 (?)	..	..	..	2
	F ₃	..	..	..	..	..	..	..	..
multiple	F ₁	..	..	..	1 (?)	..	..	..	..
	F ₂	..	..	..	..	..	..	..	..
	F ₃	..	..	..	..	1	..	..	..

markers, twenty-four flies had one or more legs affected. The "dachs" effect is probably the extreme expression of the leg abnormalities. It is therefore concluded that in these test crosses the "dachs" effect is not a mutation (gonial or somatic) but an expression of the pod effect. (It was shown for pod-G [see p. 107] that the dominant H enhances selectively the leg effect to the dachs type; dachs was also mentioned as a pleiotropic effect of pod-H.)

Flies with one wing dumpy and one wing normal appeared twice. Both gave the expected ratios of 1 normal, 1 dumpy, on backcrossing to dumpy. Although it cannot be proved that these are not somatic mutations which are nonhereditary because they did not affect the germ line, in view of the fact that podoptera has a variable expressivity with a high degree of asymmetry the explanation that the "dumpy" effect is an expression of the pod effect is more likely.

The next two, plexus-like wing and mosaic ebony, are of interest because all three flies were also Minute mosaic. These cases would suggest that a deletion may have occurred permitting the expression of the mutant. However, in the flies with ebony-like spots, the Minute and ebony patches did not coincide. Secondly, both plexus and ebony have some heterozygous expression. It may be by chance that the mutant phenotype was more strongly expressed in these particular flies.

The final mosaic, a fly with normal bristles on half of the body and shaven bristles on the other, is undoubtedly a case of elimination of the fourth chromosome.

Therefore, with the exception of the gynandromorphs and the haplo-IV mosaics, there is no evidence of chromosome elimination in the pod-H stock.

3. SUMMARY OF PART V

1. The results from reciprocal crosses show that gynandromorphism and the podoptera effect in pod-H are inherited after the manner of so-called maternal inheritance. This means that the pod-H factor works at least in part through a process taking place in the unfertilized egg. The result is apparent dominance in F_1 from a pod-H mother, recessivity in reciprocal F_1 , and identical segregation in both F_3 . The factor responsible for this is in the second chromosome.

2. Tests of F_3 progeny from individual F_2 females showed no evidence of independent assortment of gynandromorphs and podoptera. Thus, it is probable that a single factor with pleiotropic effects or two closely linked factors are responsible for the two phenotypes.

3. The production of gynandromorphs (as high as 4.0 per cent in one strain) is the result of the elimination of an X chromosome. The fourth chromosome was eliminated in a very low frequency, but there is no evidence of either chromosome elimination or somatic crossing over in the second and third chromosomes.

PART VI. DISCUSSION

Summaries of the facts are found after each of the five parts of this paper (on pp. 108, 155, 245, 265, 277).

A discussion of the phylogenetic significance of the podoptera mutants has been presented before (Goldschmidt, 1945). The question whether it is permissible to draw phylogenetic conclusions from homoeotic mutants has since been discussed. This problem will have to be taken up again in connection with the discovery of new homoeotic mutants such as the one recently reported by Herskowitz (1949). We shall not discuss it in the present paper. The same applies to the evolutionary significance. The senior author has returned to this subject recently (Goldschmidt, 1948*a*) and has discussed it sufficiently. There are other points which have already been discussed in connection with the presentation of the data, and a special discussion of the phenogenetic situation is found at the end of Part II (p. 157). The following general discussion will deal only with the interpretation of the genetic facts.

It will simplify the presentation if the hypothesis involved is stated first. The specific features of the podoptera effect make it difficult to account for them on the basis of standard formulations, e.g., by calling the genetics of podoptera just another case of multiple-factor inheritance. The peculiarities of the case are best understood if it is assumed that we are not dealing with ordinary euchromatic inheritance but that the pod factors which were analyzed are mutants of the heterochromatic sections of the chromosomes. These specific features, which are all connected with one another and must therefore be considered as a whole, are: (1) The ubiquity of genetic factors for the pod effect. (2) The grading of its penetrance to values so low that a complete absence can hardly be proved. (3) The presence of pod factors in all chromosomes. (4) The production of many different phenotypes which are typical for each line. (5) The collaboration of these factors as a multiple-factor system within each line, each with a very low individual effect, but with the effect increasing geometrically in combination. (6) The lack of allelism between the pod factors of different lines. (7) The lack of multiple-factor collaboration of factors brought together from different lines. (8) The pseudoallelism between pod factors from different lines and also from different chromosomes. (9) The pseudoallelism between pod and factors in different strains of dominant mutants. (10) The influence of pod upon the degree of dominance of specific dominants. (11) The fact that in one pod line the expression in females requires the presence of an extra Y chromosome. (12) The specific effect of extra Y material upon pod expression in all lines. (13) The fact that the dominant Bd, which is one of the dominants interacting with pod, is able to replace an extra Y chromosome in the case of pod-K referred to in point 11. (14) The ability of one of the pod factors to cause chromosome elimination (pod-H). (15) The relation of pod to the Minutes which are probably of heterochromatic nature. (16) The localization of at least one pod factor in a heterochromatic section of the first chromosome. (17) The maternal inheritance of pod-H, i.e., action upon the organization of the unfertilized egg.

Heterochromatin is mainly a cytological concept; it must therefore be asked whether the cytological facts permit or even suggest definite genetic functions of those parts of the chromosomes. It is not necessary to review here all the facts concerning heterochromatin. Recent reviews, written from different points of view, are those by Serra (1942), Heitz (1949), Schultz (1941, 1947), Caspersson (1947), Pontecorvo (1944), Kaufman (1948), Resende (1945), where the special literature is quoted. There are many obscure points as yet, and some authors present interpretations which are difficult to accept. But it is possible to select a certain group of facts which are well established and which also permit certain conclusions. These facts are the following (see presentation by Goldschmidt, 1949):

Heterochromatin, discovered by Heitz, is characterized generally by allocyclic behavior and by heteropyknosis. The first means that frequently heterochromatic sections of the chromosomes are highly chromatic (charged with nucleic acid in Darlington's terminology) when the euchromatic ones are not, and vice versa. The second term refers to the tendency of some heterochromatic section to remain as highly chromatic masses when the euchromatic sections have become invisible. Heteropyknosis is actually a special aspect of allocyclic behavior. The latter property is actually a part of the first mentioned one.

Heterochromatin is certainly not a single substance. In *Drosophila melanogaster*, which is our special interest here, at least the following heterochromatic parts can be distinguished: (1) The entire Y chromosome. (2) Blocks of different size near the spindle-fiber attachment of all other chromosomes. The Y and these blocks unite in the giant nuclei of different resting cells, especially the salivary gland cells, into a single chromocentral mass. They are most easily soluble in alkali (Kodani, 1941). (3) Intercalary blocks of definite numbers and sizes in all chromosomes. In ordinary mitosis they appear as darker staining chromomeres (Heitz). In the salivary chromosomes they are less chromatic than the euchromatic sections and their bands are less distinct. They dissolve more easily in alkali than the euchromatic sections, which permits mapping of their location on the chromosome (Kodani, 1941). The most important feature of these intercalary sections, for our present work, is their unspecific attraction. The decisive characteristic of the euchromatic parts of the chromosome, in which the typical mutant loci are generally assumed to be situated, is the point by point specific attraction which is so strong that it works even in inversions and translocations. But heterochromatic sections tend to pair with each other within or even between chromosomes. Prokofieva (1939) and Slizynski (1945) could use this for an exact localization of the intercalary heterochromatic sections. In plants, Darlington and La Cour (1940, 1941) were able to localize these sections by treatment with cold, producing what they call chromatin starvation, visible as faintly stained sections. (4) A last type of heterochromatin which has to do with the formation of the nucleolus and perhaps with that of the centromere is less well known (see Caspersson, 1947).

From the standpoint of genetics, the large heterochromatic sections were considered to be inert, not to contain genes, as the sections may be absent without causing major effects (Heitz; Muller). The intercalary heterochromatin seems to have been tacitly included in this judgment. But a number of facts are known which point to a very definite function of heterochromatin. These are the purely morphological facts which indicate a relation of heterochromatin to differentiation. Three sets of facts should be mentioned which, in different ways, all point in the same direction. The most important of these is the case of chromatin diminution in nematodes, where, in all the cells of the germ track, the chromosomes remain intact, whereas in the somatic cells the major part of the chromosome is removed and dissolved in the cytoplasm. Heitz (1935) had pointed out that Boveri's proof that the chromosome ends appear in prophase in the same position in which they disappeared in the preceding telophase is based on a characteristic feature of heterochromatin, and both White (1936) and Darlington (1932) assumed that the diminished chromatin must be "inert" material. The final proof of the heterochromatic nature of the diminished chromosome sections was furnished by Lin in our laboratory with the demonstration of all stages of heteropyknosis (Goldschmidt and Lin, 1947). This permitted the senior author to point to the fact that nematodes are cell-constant animals in which most of the somatic cells soon stop dividing and grow to large size. This could mean that heterochromatin is responsible for nuclear division and therefore is needed only in the sex cells of a cell-constant animal. One might also speculate about the immense amount of heterochromatin turned loose into the cytoplasm of the somatic cells where it could be responsible, through its ribonucleic acid, for the immense growth of these cells. The second set of observations found by Darlington and Thomas (1941) might be called the reciprocal of the first. In the presence of extra amounts of heterochromatin in pollen nuclei of *Sorghum* in the form of supernumerary B chromosomes, extra divisions take place. A third case is the remarkable behavior of the heterochromatin of the ovogonia of *Dytiscus* (Giardina, 1901). Here an ovogonium divides into 16 cells, 15 of which become nurse cells which do not divide further. All the heterochromatin of the chromocenter goes into the egg cell in the most unequal division known to cytologists. Here again, nondividing cells are left without heterochromatin, all of which is monopolized by the cell which will divide later. In this case also a relation of heterochromatin to protoplasmic growth (in the egg cell alone) is clearly visible.

Returning to *Drosophila*, it can be said that the chromocentral heterochromatin cannot play a specific role in heredity comparable to that of the euchromatin. (Most geneticists would express this by saying that this heterochromatin does not contain genes.) This must not necessarily mean that it is inert in a physiological sense. It might have a generalized function acting upon growth, cell division, differentiation. Many facts found in *Drosophila* indicate that the addition of an extra Y chromosome changes the action of genetic factors. Thus, the motting position effects are affected by an extra Y chromosome (Schultz, 1936); the extra Y also has an influence upon bristle

number (Mather, 1941, 1944) and upon the expression of a fourth-chromosome mutant (L. V. Morgan, 1947). Clearly, certain features of development and differentiation in *Drosophila* are affected by the quantity of chromocentral heterochromatin. Thus far, no experiments are available with which to evaluate possible effects of the intercalary heterochromatin, although this might be possible in translocation experiments with well-defined sections known to be mostly heterochromatic. On the basis of the cytological facts one should consider the following points: (1) Mutant loci strictly comparable to the euchromatic ones with typical allelism are not expected to occur. (2) As the heterochromatic intercalary sections are clearly integral parts of the chromosome of essentially the same structure as the euchromatic parts, one should assume that they play a role in heredity. (3) As they show such unspecificities as mutual attraction, one should expect them to be organized on a level more general than the point by point specificity of the euchromatin, with its exact polarization visible in the order of the mutant loci. Such less specific organization could conceivably be the action of an entire segment, maybe related to its quantity, or of subsegments, still large in comparison with euchromatic mutant loci. (4) The unspecificity should involve identical or similar functions or functions controlling certain generalized processes, such as those of early embryonic growth and differentiation. (5) Just as identical homologous euchromatic loci show the phenomenon of allelism, the relative unspecificity of the heterochromatic sections should make them unspecifically allelic among each other, i.e., there should be pseudoallelism among perhaps all the sections. (6) The most typical feature of euchromatic sections is the mutability of the individual loci to new alleles, which is, of course, the actual source of information we have on the difference of the loci. There is no reason to assume that the heterochromatic intercalary sections are devoid of the faculty to mutate. But in view of their unspecificity or generalized behavior, we might expect also a different type of mutation, namely, a less specific one. This would mean that mutation in these sections might be of largely the same type in all sections and produce therefore a similar effect. In order to emphasize the difference, we might use a more vague term, e.g., "mutational change" when the heterochromatin is involved.

A comparison of these expectations derived from an analysis of the cytological facts and the genetic facts enumerated above shows why it has been concluded that the pod effect is based upon mutational changes in the heterochromatin. It may be stated first that a stringent proof for this conclusion is, at least at present, impossible. Even if one succeeded in isolating segments that were undoubtedly heterochromatic (as it has been done for the large block of chromocentral heterochromatin at the base of the first chromosome) and checked upon the effect of their absence or duplication in normal and mutated condition, still a recourse to minute euchromatic insertions would be possible. Thus, today, only circumstantial proof is possible.

Point 6 of the just discussed expectations is in agreement with a considerable body of facts reported in this paper. There are distributed over all the chromosomes factors—we realize now why the general term "factors" was always used

instead of "loci" or "genes"—which essentially have the same effect in their mutant condition, i.e., the podoptera effect. In addition to this generalized or unspecific action, there is a certain amount of specificity (the typical phenotypes of the different lines, the lack of collaboration between the factors from different lines in most cases). There is, further, the amazing ubiquity of the pod factors in spite of the relative inviability produced by their accumulation resulting in selection against them. If a large number of segments, however, can show the mutational change to pod factors this is numerically the equivalent of a very high mutation rate of a euchromatic locus with negative selection only for the combinations of many mutational changes, i.e., high penetrance.

The agreement of expectation no. 5 (pseudoallelism) with the experimental facts is impressive. We found in many cases that pseudoallelism between pod factors even in different chromosomes exists. This is a rather important point.

Another suggestive group of facts are those concerning the action of extra heterochromatin. Previous authors (e.g., Schultz, *op. cit.*; Mather, *op. cit.*) have taken it for granted that a change of a genetic effect produced by the presence of extra amounts of chromocentral heterochromatin is an indication that the effect itself was based upon heterochromatin. It is a priori not certain that this conclusion is inevitable. It would be conceivable that such extra heterochromatin affects growth processes which in their changed condition might interfere with the action of euchromatic mutants as well as with the action of heterochromatic ones. But the assumption made by the authors just mentioned is strengthened considerably when facts are evaluated of the type found in podoptera. This means the case of pod-K where females are pod only in the presence of an extra Y and the huge and specific effects of extra Y's reported for tet and pod-G.

This brings the discussion to the strange fact that Bd was able to replace the action of an extra Y in pod-K, and that Bd also showed pseudoallelism with pod factors, and that twice a Bd allele appeared by mutation in a pod stock. Are these facts only coincidences? Can we conclude that Bd itself is a heterochromatic mutant? Great caution is advisable here. We reported other cases in which dominants, both presumable point mutations and position effects, interacted with pod either as pseudoalleles or as enhancers, but also the cases (e.g. Bx) in which pod factors increased the penetrance of the dominant by what also may be termed pseudoallelism (in heterozygous condition). It is possible that all these interactions are based upon a basic similarity, i.e., heterochromatic nature of the dominants. But a proof for this assumption is not yet available.

Still more difficult is the evaluation of the relations of pod factors to the Minutes. These dominant mutants (many known to be deficiencies) have much in common with the pod factors. They are distributed over all chromosomes. They all have the same general effect and only minor specific differences. The effects are similar to the pleiotropic effects of the pod factors, e.g., change of wing shape and venation, bristle effects, effects upon the legs. In addition, many of the Minutes are situated in highly heterochromatic sections of the chromosomes. But most of the Minutes are dominant and homozygous lethal

(not all, if, e.g., bobbed is to be considered a recessive Minute). The Minutes have also complete or almost complete penetrance. But there exists also one dominant and completely penetrant podoptera (Rapoport's). Altogether, one is inclined to consider the Minutes as heterochromatic effects, the more so as they affect growth rates considerably. One may safely assume that all dominant Minutes are deficiencies, although some are too small to be seen in the salivaries. Thus, the hypothesis offers itself that some small deficiencies of the intercalary heterochromatin produce the phenotype of Minutes, and another mutational change of unknown type results in the podoptera effect. The majority of the Minute stocks contain podoptera, and, in one case, the pod factor was inseparable from the Minute locus; further, all Minutes acted upon podoptera either as enhancers or pseudoalleles (which might be basically the same thing). Although all this does not amount to a stringent proof, the hypothesis seems justified that both Minutes and podoptera are different types of changes in the heterochromatin. It should be emphasized again at this point that in the virgin field under discussion only ideas based upon circumstantial evidence can be presented and offered as suggestions—a proof is not yet available.

In support of this hypothesis, the work of Mampell (1945, 1946), which shows some remarkable parallels to the podoptera work, may be mentioned. This author describes in *Drosophila persimilis* what he calls a recessive mutator. This is situated near cinnabar in the second chromosome. This mutator produces somatic mosaics, most of them of the Minute type. Sometimes, the germ cells are affected and the resulting hereditary Minutes can be attributed to loci distributed all over the chromosomes. Many of the mosaics indicate that deficiencies of different length have been induced, and in some cases that an entire chromosome has been eliminated. The percentage of somatic mutants (mosaics) is of the same order of magnitude as the percentage of mosaics found in the pod-H line. This parallels the fact that the pod-H factor produces chromosome elimination. Also, gynandromorphism is frequent, just as in pod-H. Another feature is Mampell's statement that females do not produce the mutator effect, but in XXY females the effect appears (an extra Y in males does not enhance the effect). Again, we compare this with our findings on the Y chromosome, e.g., the pod-K case, in which the females need an extra Y for the pod effect. Mampell discusses briefly the possibility also of differences between Y- and X-heterochromatin, although he does not realize that intercalary heterochromatin might have a specific genetic action. It might be added here that another strange relation has come to light which indirectly belongs to the present discussion. We have already discussed the behavior of the dominant Beaded, which showed definite relations to heterochromatin (see discussion of replacement of Y-chromosome action in pod-K, in Part III, 4, D). Therefore, both Bd and Minutes were combined in the same individuals with the result that part of the males of this combination became intersexual. The possibility that heterochromatin is involved in sex-determination has since been discussed in Goldschmidt, 1948b.

The action of the pod factors can be described as multiple factor inheritance with the added specific features which were studied in the first two parts of this paper. Certainly the details of this case of multiple factor inheritance are different from those of standard cases, but this must not in itself signify a different genetic basis. But actually a genetic analysis is on record in which a study of multiple factors (by Mather), called by him polygenes, led to the conclusion that "polygenes" are located within the heterochromatin. Mather's basic reasoning was as follows: Supernumerary chromosomes in plants known to be heterochromatic are not indispensable but exist in a kind of balanced condition and therefore must be favored by selection. This means, Mather claims, that they have a balanced polygenic action. They do not contain major genes, and they are heterochromatic. Because they are not indispensable, genes with similar action must be contained in the ordinary chromosomes. These chromosomes must contain groups of linked multiple factors or polygenes to insure both genetic stability and a possibility for genetic recombination; Mather calls them "balanced polygenic combinations." By analogy with the supernumeraries it was assumed that the heterochromatic blocks in the chromosomes are the seat of highly balanced polygenes. To prove these ideas, a character with quantitative variation—the number of abdominal chaetae in *Drosophila*—was chosen. By selection experiments their polygenic inheritance was ascertained, and certain aspects of Mather's selection experiments were assumed to show that the polygenes in question were distributed in blocks through the chromosomes, and that their balanced condition could be changed by crossing over. This polygenic action was then tested for possible interaction with the heterochromatic Y chromosome by adding Y's of different origin and marking the effect on number of chaetae. It was found that Y chromosomes of different origin exert a specific, rather small influence upon the number of chaetae, some in the minus, some in the plus direction. This effect is continuous and rather small in comparison with that of environmental influence. This action of the Y chromosome "must" depend upon a number of genes, and since polygenes in different chromosomes are known to have a similar effect, it was assumed that the Y chromosome has all the properties of a polygenic combination: quantitative action, depending upon a number of genes with similar small quantitative effects; balance of these genes; and ability of recombination. From this it was concluded that all heterochromatin carries polygenes.

A parallel with podoptera can be established in regard to the multiple-factor inheritance of the character studied, the course of the selection experiments, and the effect of extra heterochromatin, as well as the specific effect of certain Y chromosomes. As to the explanation, both sets of facts point to a location of the underlying factors in the heterochromatin. But Mather tries to transfer the gene string concept of the structure of the euchromatin to the heterochromatin, except for assembling his polygenes into blocks. A discussion of this part of his conclusions would be premature in view of the small body of facts thus far available.

A search of the literature might reveal further facts which point to the role of heterochromatin in heredity. An interesting situation was presented and discussed by Dunn and Caspari (1945). They found five different mutants affecting the mouse tail, arranged in close proximity in the same chromosome and showing some pseudoallelic behavior. They reject the explanation in terms of "repeats" as postulated by Lewis (1945) for the Star-asteroid case, and McClintock's (1944) explanation in terms of overlapping deficiencies. They are impressed by the similarity of certain features of the Minute mutants to those in a parallel case in maize; they state directly that the phenotype of the mutants must depend upon the kind of genic material which has been changed, and conclude, "It is suggested that the segment of chromosome in which these mutations occurred is so constituted that any change in it is likely to be extensive and to cause abnormalities in early developmental processes." The similarity of these data and conclusions to those on podoptera seems impressive. Thus, from all sides material is being assembled which points to an important genetic role of heterochromatin with features completely different from those of euchromatic inheritance. The "inert" heterochromatin might soon become as important as the euchromatin in genetical research.

In order to prevent misunderstanding it should be emphasized again that in the foregoing discussion the hypothesis of the heterochromatic nature of the podoptera factors and the consequent conclusions upon the genetic role of heterochromatin are meant to apply to the intercalary heterochromatin. The heteropyknotic or block heterochromatin seems to have a much less specific action, as is indicated by the purely quantitative shifts of the pod effect in a plus or minus direction produced by extra Y material. This does not, however, exclude the possibility that sections within the block heterochromatin are of the same type as the intercalary heterochromatin. One could point to the so-called normal allele of bobbed and the fertility factors in the Y chromosome of *Drosophila*. Future work might furnish the basis for better understanding.

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PLATE

PLATE 12

Fig. 1. An early stage in the development of the wingleg by enlargement and modification of the costa: Type IIc.

Fig. 2. Development of the wingleg by mirror-image duplication of the costa: IIc. Details pictured in text fig. 2.

Fig. 3. Bipartite wingleg produced by mirror-image duplication of the costa: Type Vb.

Figs. 4-5. Formation of the wingleg by duplication of wingleg anterior to the true costa: Type Va. Details in text figure.

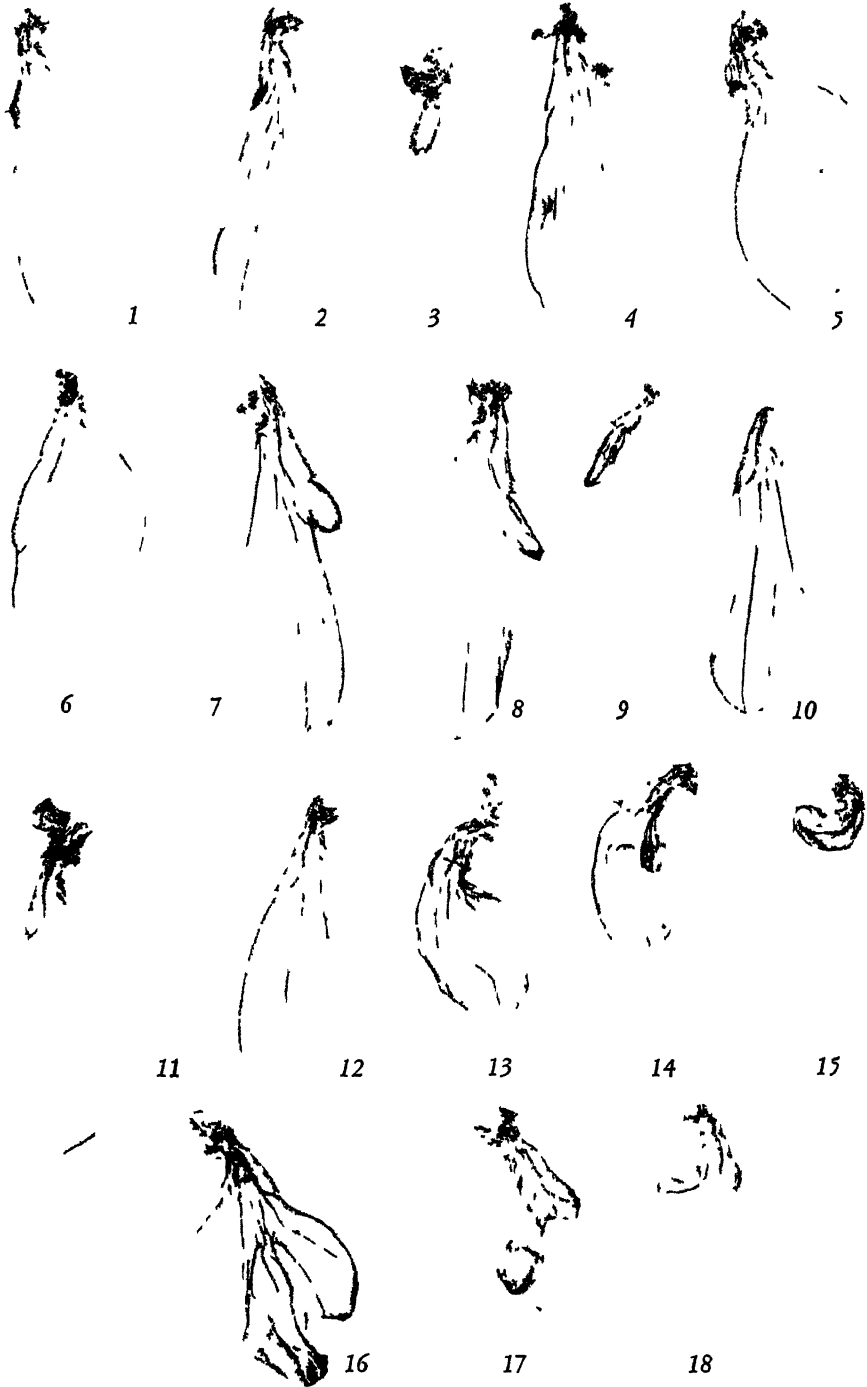
Figs. 6-8. Stages in formation of a tripartite wingleg from the costa and part of the marginal vein.

Fig. 9. Tripartite wingleg: Type V.

Fig. 10. Reduction of the wing blade by cutting: Type IIa.

Figs. 11-15. Changes in the wing blade by gradual reduction: Type II $\bar{d}$.

Figs. 16-18. Reduction of the wing blade through the vestigial-like stages.



THE LIFE CYCLE OF THE DICYEMID MESOZOA

BY

BAYARD H. McCONNAUGHEY

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THE LIFE CYCLE OF THE DICYEMID MESOZOA

BY

BAYARD H. McCONNAUGHEY

INTRODUCTION

THE DICYEMIDS are small vermiform organisms occurring in large numbers attached to the renal organs of various cephalopod mollusks. The simplicity of their morphology indicates that they belong very low in the phylogenetic scale of the metazoa, but in spite of extended studies of the group by several zoologists little agreement has been reached about their classification or life cycle. Part of the life cycle still remains unknown.

Much of the conflict of views stems from the fact that generalizations have been made from observations on particular species or a few individuals without sufficient comparative basis. This has caused confusion in the study of other species in which the phenomena differ slightly. Another source of difficulty is that the most thorough studies seem to have been made on more specialized members of the group which are in some ways less favorable for study, particularly in phenomena relating to the accessory nuclei in the axial cell of the rhombogen phase. Secondary phenomena make this particularly difficult in some species.

Rather than present here the different schemes proposed by various authors, it has seemed expedient to take up the various phases of the life cycle in order, starting with the infection of young cephalopods, to discuss each in detail, and finally to summarize the life cycle as interpreted by the author, pointing out where this interpretation agrees with and where it differs from previous accounts.

The present paper is the second part of a study of the dicyemids; the first part, the taxonomic part, has already appeared (McConnaughey, 1949). The work was conducted at the Scripps Institution of Oceanography under the auspices of the Department of Zoölogy, University of California, Berkeley. The writer is greatly indebted to Dr. Harold Kirby and the late Dr. S. F. Light of the University of California; to Dr. Dennis L. Fox, Dr. Wesley R. Coe, Dr. Carl Hubbs, Dr. Henry Kritzler, and Mr. Sheldon C. Crane of the Scripps Institution of Oceanography; and to Dr. Horace Stunkard of New York University for aid and suggestions.

INFECTION OF YOUNG CEPHALOPODS

It is not known how the young cephalopod acquires its infection. I have examined over a hundred octopuses from the littoral zone along the coast of southern California and a few dredged specimens from slightly deeper water. All of them, with the exception of one very small dredged specimen, were already infected, though the smallest ones were much less richly populated by the parasites. A number of the individuals of this series were very small and immature, weighing less than a gram. Similar results have been obtained by other investigators along the Atlantic coast of Europe, the North Sea, and the Mediterranean. Pelagic species as a rule

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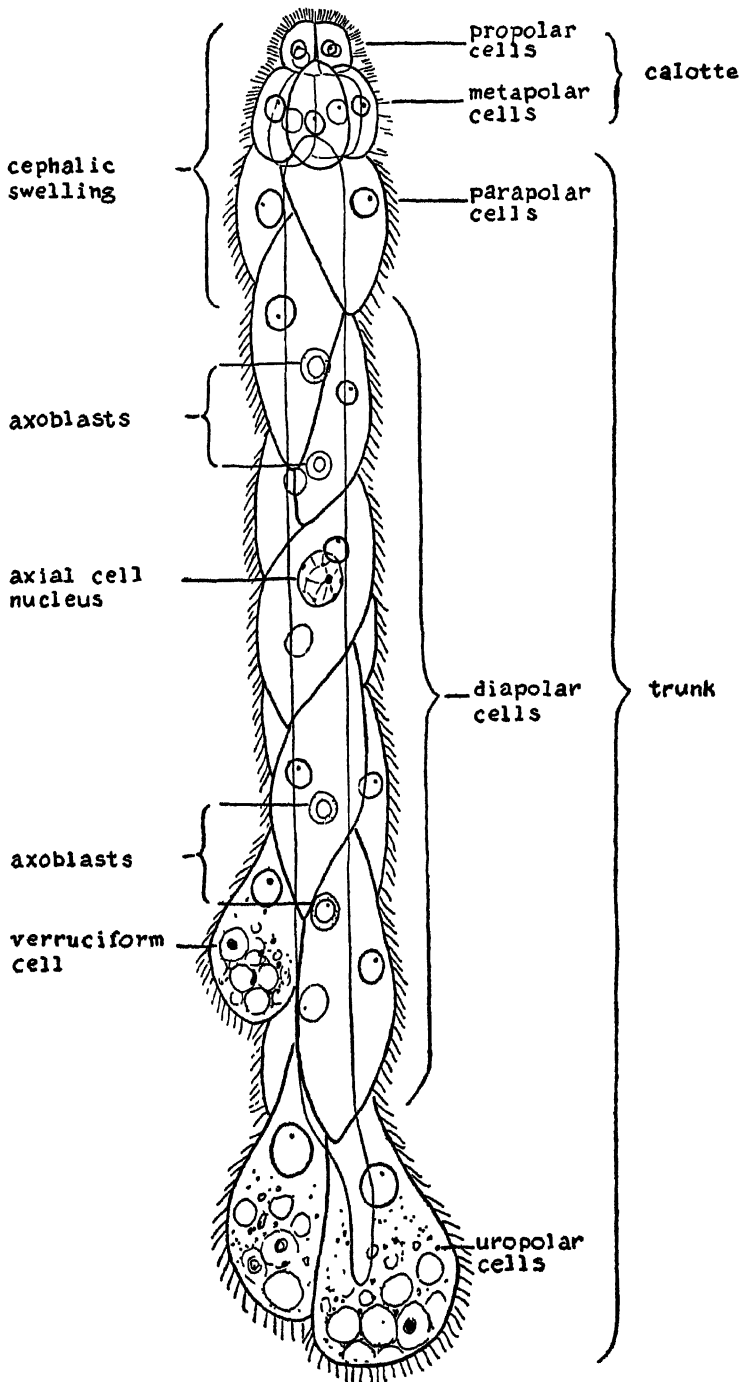


Fig. 1. Generalized drawing of a young dicyemid showing the terms that have been applied to various parts of the body. All figures in this paper, except where otherwise indicated, are camera lucida drawings from preparations in the author's collection.

are not infected. I have examined also sixteen octopuses from the reef around Oahu Island, T. H. and one from Kwajalein Island in the Marshalls. None of these was infected.

It is evident from these observations that the infecting agent is very abundant but apparently confined to relatively shallow waters. It seems not to have been introduced to certain oceanic islands separated by great distances of deep water from the nearest land. In favorable areas it appears to infect 100 per cent of the cephalopods present. Furthermore, the infection takes place very early in the life of the cephalopod, long before maturity.

If infection occurs through ingestion of an intermediate host, it would seem that this host must be very small, abundant, and itself so universally infected that none of the small cephalopods fail to acquire the parasites. The situation suggests that infection is accomplished directly from the female to her young or by some active free-swimming agent that seeks out and penetrates the cephalopod. Lameere (1916), Nouvel (1933), Gersch (1938), and I have all examined young octopuses freshly hatched from eggs in the laboratory and have found no evidence of infection in them. An opportunity for direct infection from the female to her young at the time of hatching is provided because the female deposits her eggs in recesses in the rocks and broods them until they are hatched. Meanwhile the mother is constantly liberating infusoriform larvae.

I have exposed freshly hatched octopuses and squids to large numbers of infusoriform larvae and watched them under a dissecting microscope. The infusoriforms were not attracted to the young cephalopods and no evidence of infection was found in exposed individuals with as much as a four-day exposure. Furthermore, the fact that adult male and female octopuses were both found to liberate infusoriforms at all times of the year in or out of the breeding season indicates that the infection probably does not proceed directly from one octopus to another.

The most probable method is active invasion by swarming larvae, presumably liberated from some other host. It is probable that the larval stem nematogens discovered by Lameere (1916) are the infective agents.

That additional infections often take place in the same octopus is indicated, as Whitman (1883) first clearly demonstrated, by the fact that it is not unusual to find two or even three species of dicyemids in the same individual cephalopod. When this occurs it is found that they are partly isolated from one another on different lobules of the kidney or at least in well-defined clusters, and that the predominant form is usually better developed and in a more advanced stage than the less predominant.

THE STEM NEMATOGEN

The earliest forms of dicyemid usually seen in young cephalopods are vermiform individuals, the so-called primary nematogens—nematogens that have developed directly as such from embryos without functioning as rhombogens at any previous time (fig. 1).

Lameere (1916) announced the discovery in very young *Sepia* and *Octopus* of a new phase of the life cycle of *Dicyema truncatum* and *Microcyema vespa*, preceding and giving rise to the primary nematogen. He named this the stem nematogen

(nématogène fondateur). Nouvel (1935, 1936, 1937) has described analogous forms for *Dicyema schulzianum* and *Dicyemeneae eledones* and presented additional details about those described by Lameere.

Gersch (1938) failed to find any such forms and was led to deny their existence—attributing the characteristics described by Lameere and Nouvel to faulty inter-

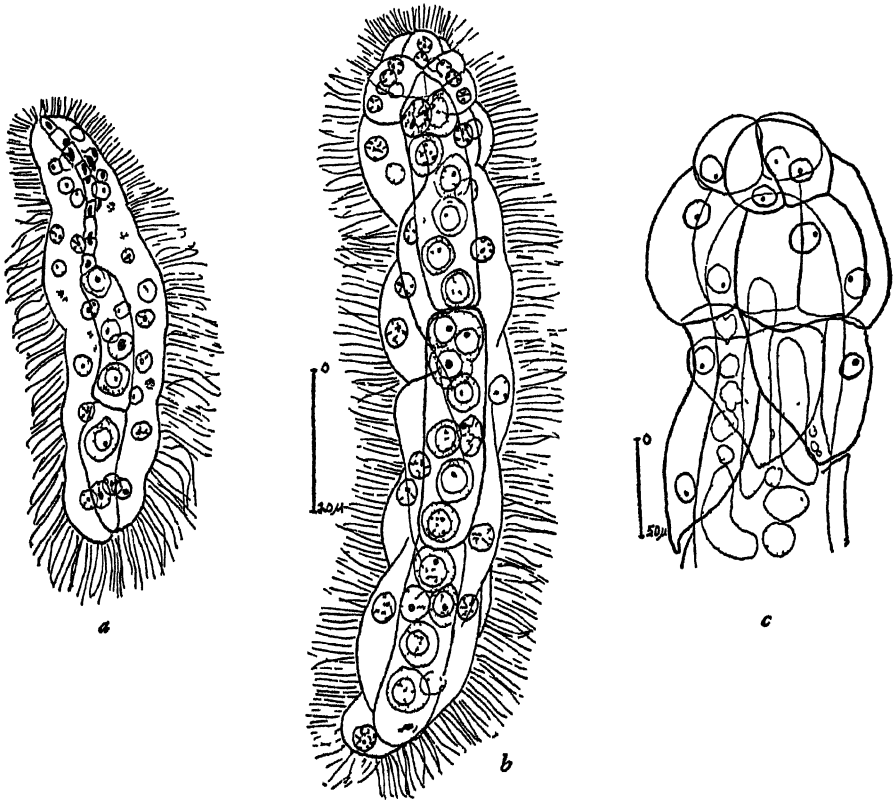


Fig. 2. The stem nematogen. a. Larval stem nematogen of *Dicyemeneae eledones*, showing a cordon of abortive cells anterior to the two axial cells. b. Young stem nematogen of *Dicyema schulzianum*, showing the three axial cells each containing several germ cells. The abortive cells present in younger larvae have been absorbed and are no longer in evidence. c. Anterior end of adult stem nematogen of *Dicyemeneae eledones*, showing the three parapar cells. Contained embryos and larvae are lightly stippled. After Nouvel.

pretation of poorly fixed material. He returned to the concept of the primary nematogen as the earliest form found in the cephalopod.

Nouvel (1947) reviewed in detail all previous work relating to the stem nematogen phase and presented some new observations of his own.

McConnaughey (1949) described stem nematogens thought to be those of *Dicyema balamuthi* from the coast of southern California.

In view of the extended treatment of the question by Nouvel it is unnecessary to repeat in detail the evidence relative to the existence of the stem nematogen. We can safely conclude that, contrary to Gersch, the forms described as stem nemato-

gens actually do exist in a number of species of dicyemids; and that they are rapidly replaced in young cephalopods by the primary nematogens to which they give rise.

The stem nematogens exhibit remarkable morphological peculiarities (fig. 2) which are of importance for a theoretical interpretation of the morphology and embryology of the other phase of the life cycle. Chief among these is the presence of more than one axial cell. The number of axial cells (three in most known species, two in one) seems constant for each species. They are arranged in linear series in the long axis of the stem nematogen. In larval stem nematogens there are in addition a series of small abortive cells, probably representing additional repressed axial cells, in front of the anterior axial cell. These cells degenerate in place and are engulfed and absorbed by the adjacent somatic cells of the young stem nematogen, leaving no trace in the older individuals. There are also differences in the number and arrangement of the somatic cells between the stem nematogens and the corresponding primary nematogens.

THE PRIMARY NEMATOGEN

The development of the primary nematogen from axoblasts in the stem nematogen or in other nematogens is the most direct and straightforward event in the life cycle of dicyemids. Yet even this has given rise to several points of difference, both in detail and in interpretation, between different authors. As the actual course of embryonic development is slightly more complicated in most members of the genus *Dicyemennea* than in *Dicyema*, the development of the vermiform larva in *Dicyema* will be outlined first. This will be followed by a discussion of the additional features characteristic of most species of *Dicyemennea*.

DEVELOPMENT OF THE VERMIFORM LARVA IN THE GENUS DICYEMA

Multiplication of axoblasts.—The germ cells or axoblasts of stem nematogens and primary nematogens multiply in the axial cell by mitotic divisions. The daughter cells are equal in size. The spindle is broad and, though converging somewhat toward the ends, does not come to a definite point. No centrosomes have been found. The chromosomes are very small and too clumped together to enable one to make satisfactory counts. Gersch (1938) described and figured for *D. typus* a stainable ring fibril between the two daughter cells that he claimed represented the remains of the spindle. He described also similar rings following cell divisions at various other points in the life cycle. Other authors have not mentioned this fibril.

Early cleavages.—Even at the first cleavage stage the difference between an axoblast entering into the formation of a vermiform embryo and one that is simply dividing is evident. The axoblast enlarges, and the first cleavage results in slightly unequal daughter cells that adhere to each other along a rather broad plane. Hartmann (1906) believed that the unequal size resulted from different growth rates of the daughter cells after division. Other authors (Whitman, 1883; Lameere, 1919; Gersch, 1938) stated that the daughter cells are unequal from the start. The latter view is correct, as the inequality is sometimes evident while division is still in progress (fig. 3, *a-d*).

The larger of the two cells remains quiescent while the smaller one and its daughter cells continue to divide. The embryo as a whole also increases in size during

this time. The cells derived from the smaller blastomere progressively envelope the larger cell which is the mother cell of the future axial cell and all the reproductive cells.

Chromatin elimination.—During the period of early cleavages some small deeply staining masses of chromatin are eliminated from the embryo. They can be seen either still in contact with the embryo or lying near by in the axial cell of the parent nematogen, generally near the anterior end in early stages, though they may occur on all sides of it. They are soon absorbed and usually are not in evidence by the time the larva has become fully developed and ciliated.

Hartmann (1906) first studied these and gave an account of their origin, showing them to be formed by an abnormal mitotic division of one of the embryonic cells, one part consisting of the eliminated chromatin similar in appearance to a polar body, the other retaining nearly all the cytoplasm. He considered this an infrequent abnormal occurrence which any of the cells of the developing embryo might experience. He further asserted that it occurred in larvae from some individual cephalopods and not in those from others, indicating that it might possibly be a pathological reaction related in some way to the condition of the host.

Lameere (1919) gave a very different account and interpretation of this phenomenon. According to him, the large future axial cell shortly before it is wholly enveloped by the somatic cells and before it forms the first germ cell, undergoes an unequal division, eliminating a tiny cell resembling a polar body. This may simply degenerate or itself redivide once or twice so that from one to four of these small abortive elements result. The axial cell itself does not give rise to any more, and, according to Lameere, not more than four of these bodies are ever found associated with an embryo.

Bearing in mind the structure of his recently discovered stem nematogen, he maintained that these small elements in reality represent rudimentary second and third axial cells together with their primary germ cells, so that the embryonic development of the vermiform larva reflects the structure of the stem nematogens but with two of the axial cells aborted—only one developing as a definitive axial cell.

This conclusion rested on the following points: 1. The abortive elements are actually derived from an early division of the large central cell of the embryo, not from any of the somatic cells as Hartmann thought. 2. The phenomenon is general rather than a rare pathological event as Hartmann thought. 3. The number of abortive elements is consistent with the number of extra axial cells in the stem nematogen. 4. They are not formed at any other stages during the life cycle.

Gersch (1938) in his account of the embryology made no mention of these small elements.

Points 3 and 4 of Lameere do not hold true. I have observed frequently more than four of these elements associated with a single vermiform embryo and have also noted their formation by young infusoriforms. This does not necessarily invalidate Lameere's general interpretation of them. Nouvel (1936, 1937) showed that larval stem nematogens have a number of abortive cells in the anterior region which seem without doubt to represent a further number of repressed axial cells, so that the actual number of these elements may not be as significant as Lameere

thought. That the contents of the urn of the infusoriforms are homologous to the axial elements of vermiform stages seems well established, hence it is not surprising that similar aborted elements might also be formed during the embryology of the infusoriform.

The formation of these elements appears to be a more constant and normal feature of the embryology than Hartmann thought, but whether Lameere's interpretation of their significance is correct is open to question.

These chromatin granules are found outside the embryos from very early cleavage stages, whereas true aborted axial cells are found within the embryos in larval *Dicycmennea* and in stem nematogens, at a somewhat later stage of development. In developing infusoriforms similar chromatin granules are derived from chromatin eliminated from only part of the blastomeres at the time of the second or third cleavages. These facts suggest another interpretation.

Goldschmidt and Lin (1947) have interpreted chromatin elimination in cell-constant animals as a phenomenon associated with the early cessation of cell divisions of the somatic cells. It has been shown in nematodes that only the sex cells, which retain almost unlimited capacity for further divisions, do not show the phenomenon, whereas the cells giving rise to the soma show early heteropycnosis of the chromosomes leading to elimination of a part of the chromatin.

The dicyemids are a classic example of cell-constant animals. The somatic cells undergo only a very limited number of divisions in the embryo, and further growth is only enlargement and differentiation of cell elements already present so that the adult consists of a small number of giant cells.

Chromatin elimination takes place during the early cleavage stages of both vermiform and infusoriform larvae but not from embryonic infusorigens of corresponding stages of development. This is very suggestive, because the infusorigen is the only stage in which there is no cell constancy, all of the cells of the individual being reproductive cells. The infusorigen does, furthermore, eliminate the paranucleus at the beginning of its formation (see p. 313). This body is here interpreted as equivalent to the small abortive second axial cell of larval *Dicycmennea*.

The formation of aborted axial cells by vermiform embryos, which in late stages of their regression give rise to pycnotic elements resembling the fragments of chromatin formed by true chromatin elimination, may sometimes complicate and confuse the picture. This is probably responsible for the diversity of opinion of earlier writers with respect to the origin of these bodies.

Formation of the first axoblast (fig. 3, *i, j, k*).—The large inner cell soon becomes wholly enveloped by the smaller somatic cells and the embryo begins to elongate, first assuming a pyriform shape. The calotte constitutes the broad end, as Whitman (1883) pointed out, and the embryo narrows posteriorly. The large internal cell divides into two unequal cells, of which the smaller has denser cytoplasm and lies posterior in position. The larger anterior cell is the definitive axial cell of the developing larva. Its nucleus becomes the axial nucleus and undergoes no further divisions. The smaller posterior cell is the first germ cell or axoblast. It promptly invades the axial cell and undergoes a mitotic division. In many species one of the daughter axoblasts migrates forward around the axial nucleus so that in larvae at this stage we find typically in the axial cell one large free nucleus, the axial nucleus, and

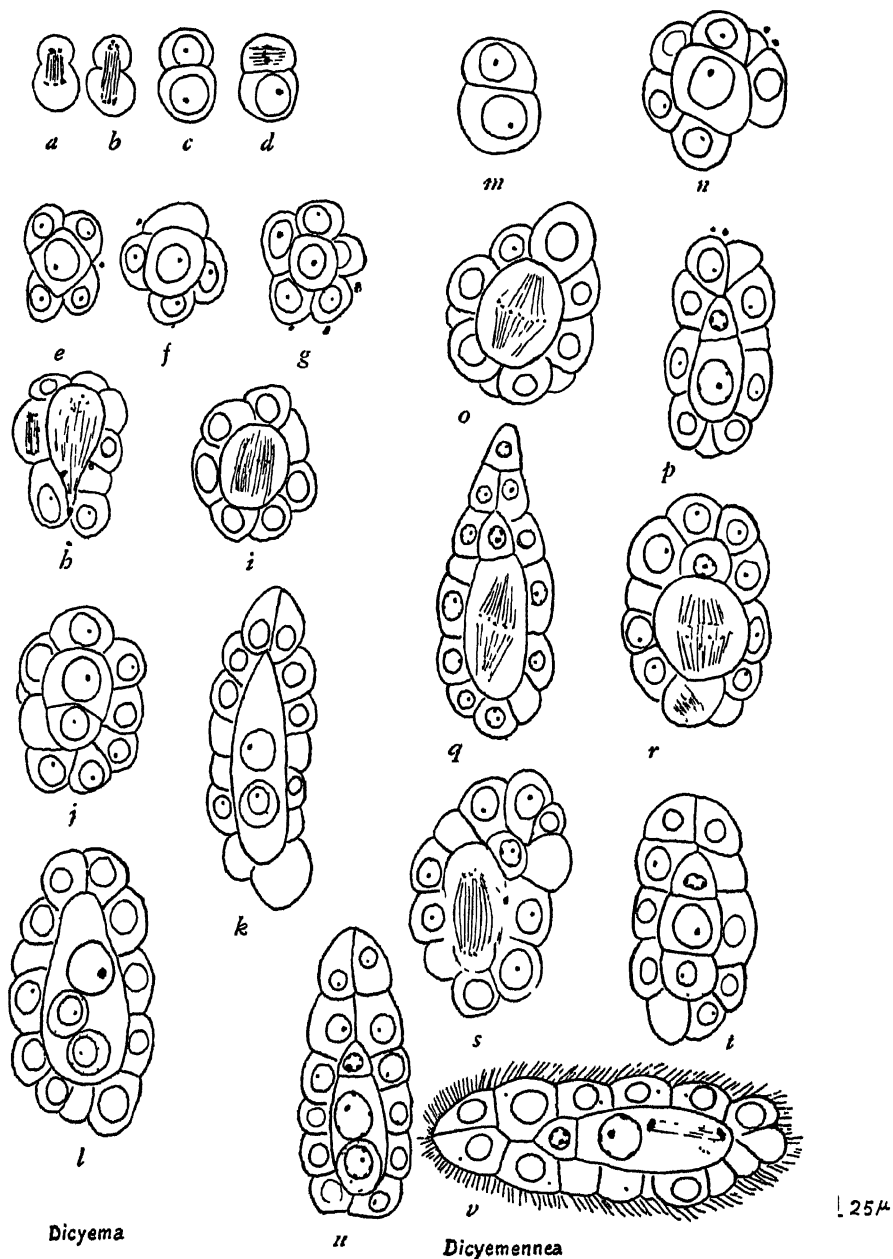


Fig. 3. Embryology of the vermiform larvae of *Dicyema* and *Dicyemennaea*. a-l, *Dicyema bala-muthi*; n-q, and s-v, *Dicyemennaea abelii*; m and r, *D. californica*.

Dicyema: a-c. First cleavage of an axoblast at the beginning of the formation of an embryo, showing the inequality of the blastomeres. d. Beginning of second cleavage of the smaller (somatic) cell. e, f, g. Optical sections of young embryos in which the somatic cells have almost completely enveloped the central cell. Some chromatin elimination has taken place. h. Optical section of an embryo beginning to elongate. This embryo seemed to have just given off an abortive element from the axial cell, supporting Lameere's thesis that abortive elements may be formed in this manner. Other granules of eliminated chromatin are, however, already around much earlier embryos before

two axoblasts, one anterior and one posterior to the axial nucleus. All further axoblasts of the larva are derived from these.

Straightforward as this development is, diverse accounts of it have been given.

Van Beneden (1876) believed that the axoblasts arise spontaneously *de novo* in the axial cell, not from division of previous cells. Whitman (1883) showed that axoblasts do not arise *de novo*. He gave an essentially correct account of the origin of the first germ cell but was not sure whether it was actually cut off from the axial cell at first, later invading it, or if simply formed endogenously by condensation of denser cytoplasm around one of the nuclei of the two-nucleated axial cell following mitosis without cell division. He also thought the second axoblast was derived from the axial nucleus by a second division, though he had not been able to trace its origin definitely.

Hartmann (1904) correctly described the derivation of the second axoblast from the first rather than from a second division of the axial nucleus. He pictured a heteropolar spindle in the first division, the germ cell going to the small (posterior) end, which he mistook for the anterior end.

Lameere (1916) described the division of the nucleus of the internal cell as occurring with a heteropolar spindle, forming two nuclei of different sizes but without any division of the cell body. Vacuolization of all the cytoplasm except that immediately around the germ cell nucleus was said to be responsible for the isolation of the axial nucleus and of the germ cell within the axial cell. Later (1919) he corrected this, having observed that the first division resulted in two separate cells followed by the invasion of the axial cell by the first germ cell. The heteropolar spindle was again pictured.

Gersch (1938) confused the problem by returning to the concept of endogenous formation of the first axoblast. He maintained that what Hartmann (1904) and Lameere (1919) took for a cell wall between the two was in reality simply a ring fibril, a remnant of the spindle plate, and that there was no separation of the first germ cell followed by its intrusion into the axial cell.

I have examined a very large number of embryos in early stages of development in several species and can say definitely that Gersch's account is erroneous in this respect. I have not observed any clear-cut cases of the heteropolar spindle as yet but I am not prepared to say that it may not occur in some species in view of the very clear statements and figures of Hartmann and Lameere about it.

any indication of a division of the central cell. *i*. Metaphase of the division of the central cell. *j*. Optical section of a larva after completion of the division of the central cell, showing the definitive axial cell and a smaller posterior cell representing the primary germ cell or first axoblast. *k*. Optical section of young larva in which the first axoblast has invaded the axial cell. *l*. Optical section of larva showing the two axoblasts resulting from mitosis of the first axoblast, before the migration of one of them to a position anterior to the axial nucleus.

Dicyemeneae: *m*. Two-celled embryo. *n*. Optical section of a young embryo, the central cell not wholly enveloped by somatic cells. Granules of eliminated chromatin present at the periphery. *o*. Optical section of young embryo showing the first division of the large central cell. *p*. Optical section of larva after first division of the large central cell. *q*, *r*. Optical sections showing second division of the large internal cell. *s*. Late anaphase of second division of the internal cell. In this embryo some chromatin seemed to have been eliminated, at the time of this division, from the nucleus destined to become the axial cell nucleus. This has not been confirmed in other larvae and is probably not a general phenomenon. *t*. Optical section after completion of the second division of the internal cell, showing a row of three cells axial in position. *u*. Optical section of larva showing the invasion of the definitive axial cell by the primary germ cell. *v*. Optical section showing the first division of the primary germ cell.

THE ABORTIVE SECOND AXIAL CELL IN LARVAL DICYEMENNEA

(Fig. 3, *m-v*)

The embryology of the vermiform larvae of most species of *Dicyemennca* differs from that of *Dicyema* in one important respect, which for some reason was entirely overlooked until 1938 when Nouvel published a preliminary note regarding it.

In vermiform embryos of most species of *Dicyemennca* the large internal cell, after it is enclosed by the smaller somatic cells, undergoes two unequal divisions instead of one as in *Dicyema*. The first cuts off a smaller cell that comes to lie anterior to the axial cell in the region of the metapolar cells. The second cuts off a similar but slightly larger cell posterior to the axial cell. Thus we have in the young embryos a row of three internal cells very suggestive of the condition of some stem nematogens (fig. 3, *l*). The largest of the three, the central one, becomes the definitive axial cell of the larva. The posterior one, as in *Dicyema*, invades the axial cell and constitutes the first axoblast. The small anterior cell, however, remains in place anterior to the axial cell between the metapolar cells, in some species near their basal ends, in others shoved well forward against the base of the propolar cells. It degenerates in place and is absorbed, leaving no trace in the adult.

In the last stages of degeneration this cell is often ingested by the dorsal metapolar cell, where it is rapidly digested within a vacuole. In *D. brevicephala* it passes into one of the propolar cells. In any case it has usually, though not invariably, completely disappeared by the time the larva escapes from its parent nematogen, or soon thereafter. *Dicyemennca gracile* (Wagener) is the only species of *Dicyemennca* in which no small abortive cell is formed during the early development of the vermiform larva.

The position and staining characteristics of this small cell suggest that it represents a second axial cell, the development of which is ordinarily repressed. An unexpected confirmation of this view was found in the case of one large abnormal nematogen with a hereditary malformation, in which the factors responsible for arresting cell division and development in the anterior region evidently suffered delay. The nematogen and two of its contained larvae showed an extra ring of metapolar cells in the calotte. The small abortive cell, present in its usual form in most of the embryos, had in these elongated and grown back, developing into an unmistakable axial cell, pushing the fore end of the regular axial cell to one side so that the larva bulged out to give almost the appearance of fused lateral twins (fig. 4).

The embryology of vermiform larvae of *Dicyemennca* reflects the condition of the stem nematogen, and probably of the ancestors of the dicyemids, more closely than does that of *Dicyema*. A small suppressed second axial cell is formed as a constant feature in most species. In *Dicyema* this is omitted, together with the fifth metapolar cell. In rare cases larvae have been found within *Dicyema* nematogens in which these were present, making the larvae typical *Dicyemennca*. It seems that the responsible factor is probably an alteration in rates of embryonic development so that the factor or factors responsible for arresting cell division in the region of the calotte are operative slightly earlier in *Dicyema*. The transition apparently can be made in one step.

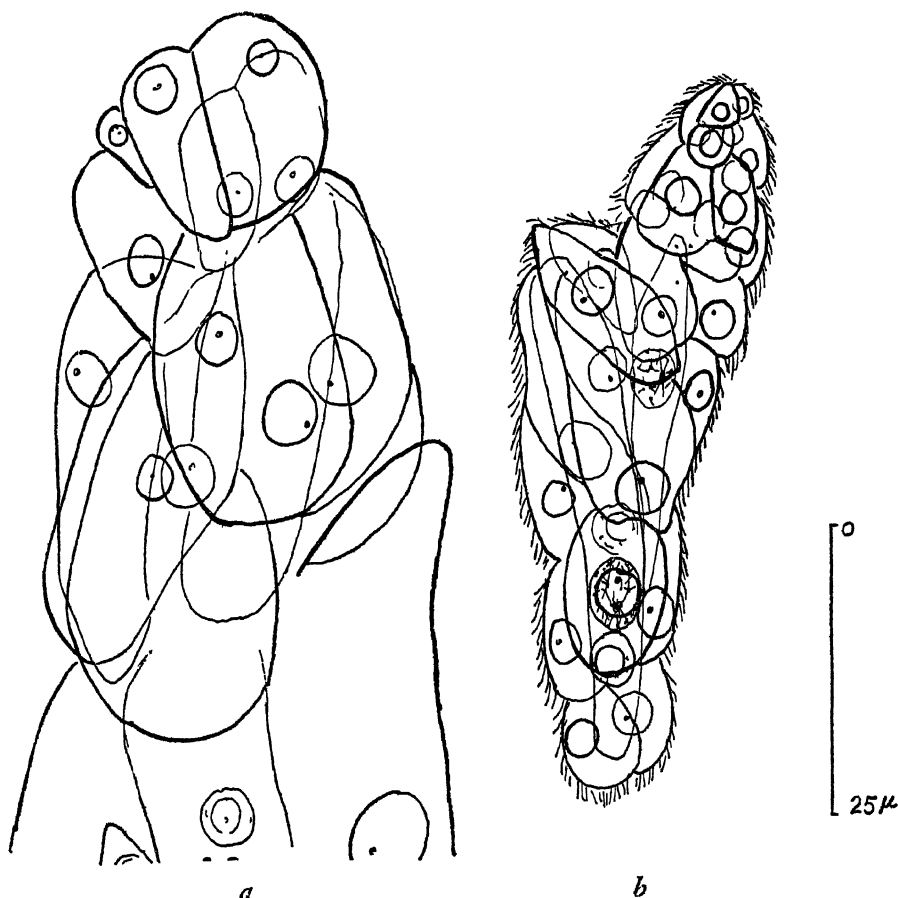


Fig. 4. *a*. Calotte of very abnormal nematogen of *Dicyema balamuthi* (?) showing almost complete reduplication of the metapolar set of cells. *b*. Abnormal vermiform larva from this nematogen, showing the same anomaly. The abortive second axial cell has here grown back and developed into a clear-cut axial cell but without any axoblasts. The anterior end of the definitive axial cell has been pushed to one side, causing a bulging. It is noteworthy that the abnormally developed calotte should be accompanied by the appearance and unusual development of this rudimentary second axial cell not usually found in *Dicyema*. Other more normal appearing larvae contained in this same nematogen were still abnormal for *Dicyema* in having five, instead of four, metapolar cells and in having a small second axial cell present in the form characteristic for *Dicyemonea*. These larvae and any adults that might develop from them would, by themselves, be indistinguishable from normal individuals of *Dicyemonea*.

LATER DEVELOPMENT OF THE LARVA

After attaining their definitive cell number and organization the larvae continue to grow simply by enlargement and elongation of existing cells. No further cell divisions take place except for multiplication of the axoblasts, which begins about the time the larvae escape from the parent nematogen. They become ciliated and active while still within the axial cell of the parent, finally escaping through the side of the body, leaving no scar. The larva fixes itself to the renal tissue of the host and grows into a mature nematogen which produces further generations of larvae. This continues until the renal organs are thickly populated by parasites.

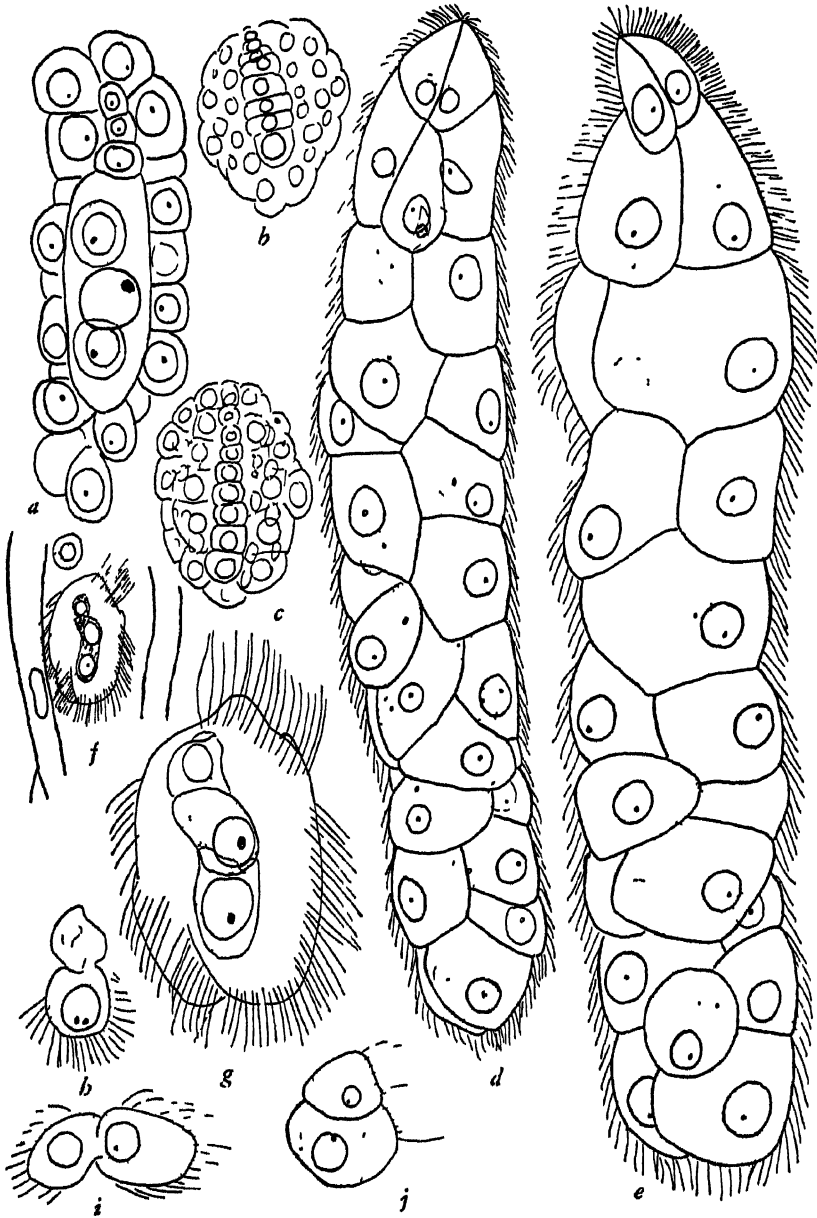


Fig. 5. a. Abnormal larva of *Dicyemenna granularis* with three abortive axial cells anterior to the definitive axial cell. b, c. Very unusual larvae of *D. granularis* with more than the normal number of cells. Internally there is a row of cells, axial in position, decreasing gradually in size toward what is taken for the anterior end. d, e. Fully formed larvae of *D. californica* without any axial cell, and with abnormal calotte in one case (e). These larvae had already escaped from the axial cells of their parents and were free swimming. f. Unusual body found in axial cell of a mature nematogen of *D. abelsi*. g. Detail of body shown in f. h, i, j. Other unusual cells scattered near by in the axial cell of the same nematogen. These appear to have been derived from the dis aggregation of a fully formed larva before its escape from the axial cell.

ABNORMALITIES OF VERMIFORM STAGES

In rare instances abnormalities occur in the vermiform individuals that throw light on related normal conditions or that may be very puzzling when first encountered. Some of those encountered during this study are listed here.

1. Abnormal numbers of cells in the calotte have already been discussed in connection with Wheeler's genus *Dicyemodeca* (McConnaughey 1949)

2 In one instance, a vermiform larva of *Dicyemennea granularis* had three small abortive axial cells anterior to the definitive axial cell instead of the usual one. This is reminiscent of the conditions described by Nouvel (1936, 1937) for very young stem nematogens (fig. 5, a).

3. One large *D. granularis* nematogen of normal appearance contained four very exceptional larvae with plump rounded bodies composed of many more than the usual number of somatic cells arranged partly in longitudinal rows, and with a cordon of eight or nine small cells axial in position and diminishing in size anteriorly. Several other cells also seemed to be in the interior of the body rather than constituting part of the surface. These larvae might be interpreted as reversions almost to an orthonectid condition (fig. 5, b, c).

4. In rare instances larvae are formed with no axial cell. They may attain full development, become ciliated, and escape from the parent nematogen. It is not known whether this condition is a result of an accidental separation of the blastomere representing the axial cell from the rest of the embryo in the earliest stages of cleavage, or if this cell may have been present in the young embryo and failed to develop further. The fact that in one instance a small rudimentary axial cell similar to that normally present in larval *Dicyemennea* was present suggests the latter alternative for at least some cases. No adults exhibiting this abnormality have been found (fig. 5, d, e).

5. In rare instances vermiform (also infusoriform) larvae after reaching full development within the axial cell of their parent, fail to make their escape. Instead, they degenerate in place and undergo complete disaggregation and absorption within the parent organism. During this disaggregation their cells, or groups of cells, may become scattered some distance up and down the axial cell, forming bizarre-appearing ciliated bodies like peculiar ciliates or other organisms wholly foreign to the normal contents of the axial cell. This disaggregation usually affects only one or two larvae in a given nematogen; others in the same state of advancement and even right beside them in the axial cell remain perfectly normal and unaffected (fig. 5, f-j).

6. Several dicyemids from one cephalopod exhibited degenerative changes in one or two of their somatic cells. The cytoplasm of the affected cells took a strong basophilic stain in sharp contrast to the other cells, and the nuclei appeared pycnotic. Usually only one cell in an individual was so affected, the others appearing perfectly normal.

THE RHOMBOGEN PHASE

When the renal organs of the cephalopod have become heavily populated by the parasites, a change of phase occurs in many or most of the individuals present. They cease producing vermiform larvae and instead give rise to a few peculiar,

reduced individuals called infusorigens that never leave the axial cell of their parent. The infusorigens proliferate a number of large egg cells that come to lie free in the axial cell of the rhombogen and from which a very different sort of larva, the infusoriform, develops.

During the period of transition numerous secondary phenomena occur in the transforming nematogen so that the rhombogen differs in its own right from the nematogen in addition to possessing a changed type of reproductive element and larvae.

Vermiform larvae liberated during this period, upon attaining maturity usually form infusorigens and infusoriforms directly, without previously functioning as nematogens. These are termed primary rhombogens. Those resulting from the transformation of older nematogens are called secondary rhombogens. There has been little agreement between different authors either on the details or on the interpretation of the phenomena attendant upon the change of phase.

For convenience, these changes are divided into several categories and considered separately, as follows:

Somatic changes differentiating the rhombogen from the nematogen.

- (a) Size and proportions.
- (b) Accessory nuclei in the somatic cells.
- (c) Verruciform cells.

Changes in the contents of the axial cell.

- (a) Degenerative changes leading to destruction of germ cells.
 - (1) Degeneration in place in the axial cell.
 - (2) Entrance of germ cells into the somatic cells and their absorption there.
 - (3) Temporary lodgment of germ cells between the axial cell and the somatic cells.
 - (4) Ingestion of some germ cells by other germ cells.
 - (5) Origin of free nuclei other than the paranelei.
- (b) Changes leading to the formation of the infusorigen.
 - (1) Fusiform and binucleate axoblasts.
 - (2) Enlarged germ cells—mother cell of the infusorigen.
 - (3) Elimination of the paraneucleus.

SOMATIC CHANGES

Size and proportions.—Rhombogens of many species tend on the average to be somewhat longer and wider than the nematogens, though this is by no means invariable. The elongation does not affect the propolar cells but may affect the other cells of the cephalic swelling so that this region becomes elongated and narrower, no longer presenting a swelling (fig. 6, *a*). This phenomenon is not constant even in the same species and is not invariably associated with the rhombogen condition. In a few species, very short thick rhombogens are found, much shorter than the corresponding nematogens. In most species the rhombogen seems more robust or heavy-set than the nematogen.

Accessory nuclei in the somatic cells.—The most conspicuous somatic change and the one that can be most surely relied upon to indicate a change of phase is the appearance of accessory nuclei in some of the somatic cells of the trunk. This takes place through an amitotic division or budding of the nuclei in these cells. The divisions are usually very unequal. Sometimes redivision or renewed budding

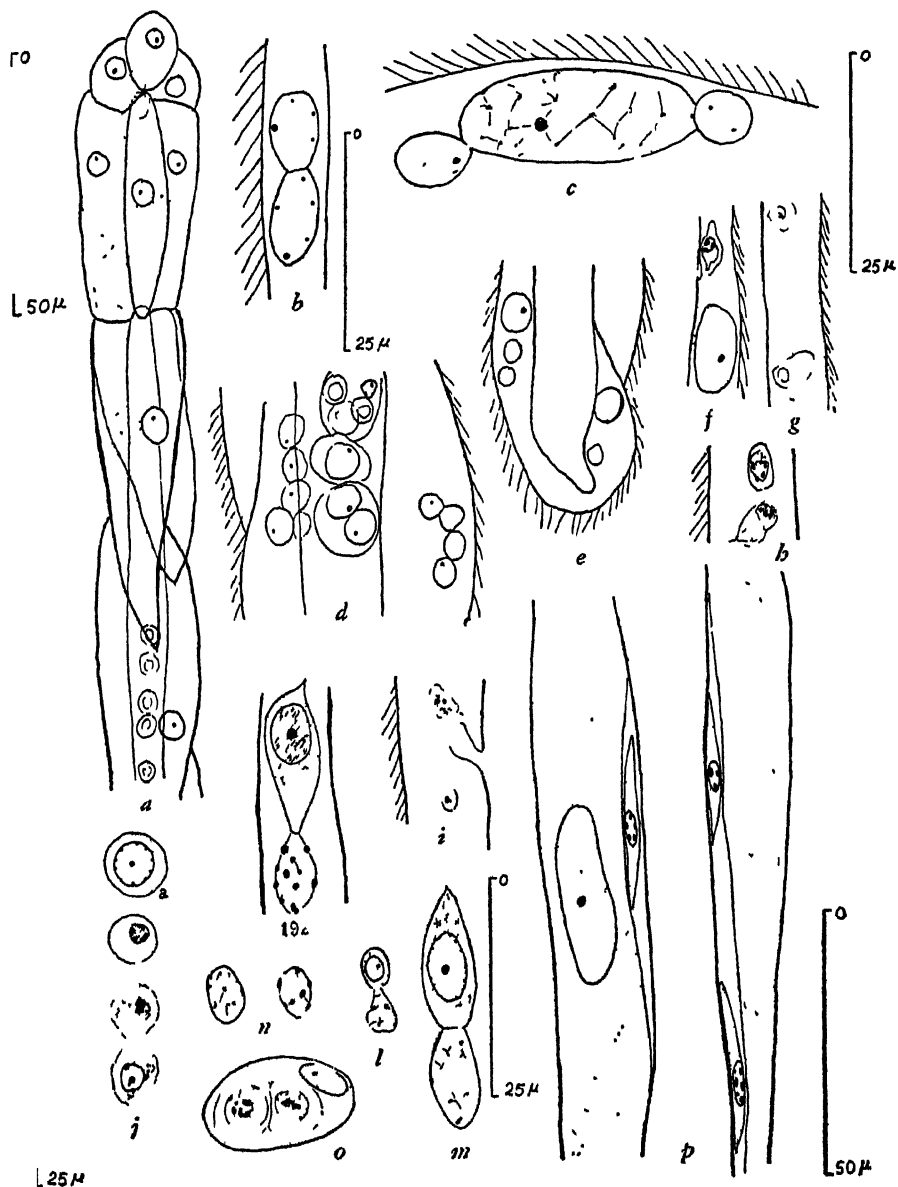


Fig. 6. Secondary phenomena attending the change of phase from nematogen to rhombogen. *a*. Anterior end of young rhombogen of *Dicyemella abelii* showing elongation of meta and parapolar cells. *b*, *c*. Amitotic division of the nucleus in somatic cells of transitional individuals of *D. adscula*. *d*. Part of trunk of rhombogen of *Dicyema acciacciatum* with little chains of nuclei in the somatic cells. *e*. Caudal end of rhombogen of *D. acciacciatum* showing accessory nuclei in somatic cells separated from the main bodies of the original nuclei. *f*, *g*, *h*, *i*. Parts of somatic cells from transitional individuals of *D. balamuthi* showing digestion and degeneration of invading axoblasts. *j*. Stages in the degeneration of axoblasts in place in the axial cell of a transitional individual of *D. balamuthi*. *k*, *l*. Elimination of nucleus-like body from enlarged germ cells in *D. balamuthi*. These bodies are apparently not formed by any direct division of the nucleus of the cell. This is what Hartmann (1906) termed the chromidial origin of accessory nuclei. *m*. Similar expulsion of a nucleus-like body from a small axoblast. *n*. Free nucleus-like bodies in the axial cell of a transitional individual, probably derived from axoblasts in the above manner. *o*. Enlarged germ cell in transitional individual of *Dicyemella californica*, containing two smaller axoblasts in the course of digestion in vacuoles in its cytoplasm. *p*. Part of trunk of an early transitional

occurs before the first bud is completely separated from the parent nucleus, so that little chains of three or four or more incompletely separated nuclei may be formed (fig. 6, b-e).

The presence of these accessory nuclei makes it difficult to obtain reliable cell counts on rhombogens. They also persist and may be seen in secondary nematogens where they constitute one of the criteria for recognizing the latter.

The time of onset of these amitotic nuclear divisions in the somatic cells does not always correspond precisely with the first noticeable changes in the reproductive elements of the axial cell. In some species of *Dicyema* in particular it may precede these changes so that accessory nuclei can often be found in the epithelial cells of mature nematogens.

Wheeler (1897) and Hartmann (1904) observed these supplementary nuclei and thought that they were derived from germ cells that passed into the somatic cells and were digested there, leaving only the nucleus.

Lameere (1918) stated that he had never observed this supposed passage of germ cells into the peripheral cells, and, from the similarity of the accessory nuclei to the regular ones present, concluded that they were formed by unequal amitotic division of the latter. Later (1919), after finding evidence of the passage of germ cells into the somatic cells, he reversed his opinion, deciding that the supplementary nuclei were derived from immigrant germ cells after all, though he stated that it was a very remarkable circumstance that the somatic cells should be able to adopt nuclei not their own and use them as such.

Nouvel (1933, 1934) pictured the entrance of germ cells into somatic cells, particularly the uropolars and verruciform cells, and their subsequent digestion there.

Gersch (1938) found amitotic divisions of somatic nuclei and considered them degenerative changes preceding the death of the old rhombogens. Nouvel (1947) agreed with Lameere that these nuclei are derived from axoblasts that have invaded the peripheral cells and whose cytoplasm has there been digested. He wrote, "L'origine des noyaux accessoires de la cellule axiale sera traitée plus loin [chapitre V]. Il paraît bien que ceux des cellules périphériques ne résultent jamais d'une division directe ou indirecte de ces cellules."

I have many times observed the presence of axoblasts in various stages of degeneration and absorption in the somatic cells of the trunk (fig. 6, f-i), particularly in individuals transitional between the nematogen and the rhombogen phases. These axoblasts, however, are always in vacuoles, and all stages of their degradation, from whole ones just entered to small deeply staining pyrenotic nuclear fragments, can be found. There is no evidence that their nuclei ever persist as accessory nuclei of epithelial cells into which they have entered. The accessory nuclei of these cells are derived from the epithelial cell nuclei themselves by amitosis or buddings. These are to be considered degenerative changes, as Gersch (1938) maintained. The change occurs, however, not as a final pathological change immediately preceding the death of old rhombogens as Gersch thought, but at the time of, or even slightly before, the onset of the rhombogen phase. Furthermore, in different species it takes place characteristically in slightly different ways and begins in different cells. Thus in *Dicyemenna granularis* the first accessory nuclei are normally formed in the parapolar and anterior diapolar cells after the nuclei

of these cells become irregular and polymorphic in shape. In several other species accessory nuclei are first formed in the diapolar cells more posterior in position along the trunk and seem to be simply budded out and pinched off from the cell nucleus without its first undergoing marked changes in shape.

Verruciform cells.—Nouvel (1933) described the formation of verruciform cells in *Dicyema clausianum* and stated that: "L'un des premiers phénomènes qui marquent la transformation du nématogène primaire en rhombogène (phénomènes rhombogéniques, Lameere) est précisément la formation des verrues..."

This statement possibly is true for the majority of individuals of some species but is certainly not a general or invariable criterion of the transition of phase. Some species do not form verruciform cells at any time. In others the two caudal cells, the uropolars, are typically verruciform in character in both phases. The single granular uropolar cell of the stem nematogen described by Lameere is of this character. In several species of *Dicyemene* I have noted verruciform cells on young nematogens as well as older ones and rhombogens.

The formation of verruciform cells seems to be a phenomenon associated with intense activity within the axial cell in the production of both vermiform and infusoriform embryos. Because the accumulation of nutritive reserves in the somatic cells preceding the active rhombogen activity is marked, and because the production of infusoriforms seems as a rule to proceed at an accelerated rate compared with that of the vermiform larvae, it may be true that verruciform cells are found more often on early rhombogens than on other stages, in those species that ordinarily have them.

CHANGES WITHIN THE AXIAL CELL

Degenerative changes leading to destruction of germ cells.—Mature nematogens, just before the transition to the rhombogen phase, often have a very large number of axoblasts in the axial cell. The majority of them are eventually lost through degeneration and absorption, only a few persisting, some becoming the mother cells of the infusorigens.

The degeneration is first evidenced by altered staining reaction of the cell. The cytoplasm becomes paler and more eosinophilic. The nucleus shows progressive clumping of the chromatin, which forms deeply staining masses at the periphery of the nucleus. The nucleus itself becomes smaller, moves to the periphery of the cell, and eventually disassociates into small fragments that may persist for a time after most or all of the cytoplasm has been absorbed. During the later stages of this degeneration the axoblasts are inclosed in a digestive vacuole in the axial cell.

The appearance of these cells during their digestion is varied, especially in the genus *Dicyema*, and at one time led Lameere (1914) to believe that it was a process of spermatogenesis. His article, *Le mâle des Dicyémides*, was based upon this interpretation. Later (1918) he realized his error and recognized that what he had taken for males were simply small groups of degenerating axoblasts in various stages of degeneration, and that what he had taken for sperm were the last pyknotic chromatin remnants of their nuclei.

Some of the axoblasts become amoeboid and invade the somatic cells, where they undergo digestion in vacuoles in the same manner as in the axial cell. When ver-

ruciform cells are present the axoblasts enter these most often, though they sometimes invade other trunk cells as well.

Less frequently a number of them may, instead of being digested in place or passing directly into an epithelial cell, become lodged between the axial cell and the inner wall of a somatic cell. Here they assume a flattened irregular or elongate, fusiform, shape and may persist for some time. Koeppen (1892) described certain amoeboid cells that he thought wandered out of the dicyemid and might perhaps be amoeboid sperm, entering others and fertilizing them. He also described muscular elements. It seems probable that what he observed was the passage of axoblasts into somatic cells and the lodgment of some between the axial cell and the somatic cells. When thin and elongate in shape these latter might easily be taken for muscle cells, and their position at this time, if we consider the axial cell endodermal as most earlier writers did, is actually mesodermal as might be expected of muscular elements (fig. 6, *p*).

A few instances of axoblasts being engulfed and digested by larger germ cells have also been observed (fig. 6, *o*).

In some cases nucleus-like bodies are eliminated from some of the germ cells in the manner described by Hartmann (1907), which he termed the chromidial origin of accessory nuclei (fig. 6, *l-n*).

Changes leading to the formation of the infusorigen.—Frequently in early transitional individuals, a small number of the axoblasts present are slightly larger than the average and possess two nuclei. Gersch (1938) and others have noted these binucleate axoblasts, though without correlating them with any particular phase of the life cycle, and have believed them simply the result of abnormal mitosis in which the cytoplasm failed to divide after nuclear division.

The uniformity with which they appear, particularly evident in young primary rhombogens where the end members of the row of axoblasts on either side of the axial nucleus are commonly binucleated (McConnaughey, 1949, pl. 2, fig. 24), suggests that the phenomenon may have a more fundamental significance. Occasionally a cell may be found in which the two nuclei appear to be undergoing fusion. Slightly older individuals show the presence of enlarged uninucleate cells, some of which become fusiform in shape with long drawn out, sharply pointed ends (fig. 7, *b*). It seems probable that some of the smaller ordinary axoblasts also become fusiform, for intergradations between these and the fusiform cells can also be found. The number of fusiform cells present often far exceeds the largest number of binucleate axoblasts seen or the number of infusorigens later formed. Many of them are lost through degradation in the axial cell in a manner comparable to that of the ordinary axoblasts. Sometimes the cytoplasm appears to be digested away, leaving only the empty cell wall and a somewhat enlarged nucleus which fills it so that the whole cell takes on the appearance of an accessory free nucleus in the axial cell. This is more often observed at the end of the rhombogen phase at the time partly formed infusoriforms are disaggregating than at the beginning.

That the binucleate axoblasts actually are precursors of the mother cells of the infusorigens has not been shown. It was suggested on circumstantial evidence and as a possible explanation for the diploid constitution of the infusorigen (Mc-

Connaughey, 1948). If the interpretation of the life cycle here developed is correct this would seem improbable on theoretical grounds as it would involve a reduction of the chromosome number to haploid sometime during the unknown part of the life cycle, as well as during the formation of germ cells by the infusorigen.

It may be, as Gersch believes, that the binucleate axoblasts represent simply another indication of the upset of normal axoblast development attendant upon the change of phase and are merely cells in which cytoplasmic division has failed to follow nuclear division.

The paranucleus.—The first definitive act of the mother cell of an infusorigen is an unequal division with the elimination of a smaller cell, which soon loses all appearance of possessing any cytoplasm and remains in the axial cell of the parent rhombogen as an accessory free nucleus—the paranucleus of Whitman (1883). It is small at first but usually increases in size as the infusorigen matures. In some species, for example, *Dicyemenea californica*, by the time the infusorigen is mature and functional, the paranucleus has attained approximately the same size and appearance as the axial nucleus of the parent rhombogen, from which it may become difficult or impossible to distinguish it with certainty (fig. 7, e-l).

Thus, typically, there comes to be in the axial cell of a rhombogen one more free nucleus than there are infusorigens—the original axial nucleus, plus one paranucleus corresponding to each infusorigen.

In some species, especially in certain *Dicyema*, the picture may be complicated by secondary amitotic divisions of the axial nucleus or of one or more of the paranuclei, or by the chromidial formation of free nuclear bodies as described by Hartmann (1906). In such cases the number of free nuclei in the axial cell no longer is equal to the number of infusorigens plus one but exceeds this by an indefinite number.

These secondary phenomena, together with the budding off of accessory nuclei in the somatic cells, have confused the picture to such an extent that some authors have been led to deny that there is any relationship at all between the infusorigens and the free nuclei present in the axial cell.

Gersch (1938) for instance, says: "Wie aber schon Hartmann, Lamcère, u. a. gezeigt haben, handelt es sich dabei um Verwechslungen mit den Kernen von Ausenzellen und mit Fragmenten des Axialzellkerns, wie sie in alternden Würmformigen nicht selten vorzufinden sind."

It seems odd that Gersch should cite Lamcère in support of this point of view, when Lamcère himself (1919), after further study of the problem, renounced his earlier position (1918) and gave a detailed account of the origin of the paranucleus corresponding closely to that of Whitman.

It is true that other accessory nuclei sometimes arise in certain species in the manner described by Hartmann (1904), Lamcère (1918), and Gersch (1938). But this in no way invalidates Whitman's general thesis about the origin of the paranuclei though it does lessen the value of accessory nuclei as criteria for recognition of secondary nematogens.

In several species of *Dicyemenea* the phenomenon is usually uncomplicated, the true paranuclei being the only accessory nuclei present in the axial cell, and

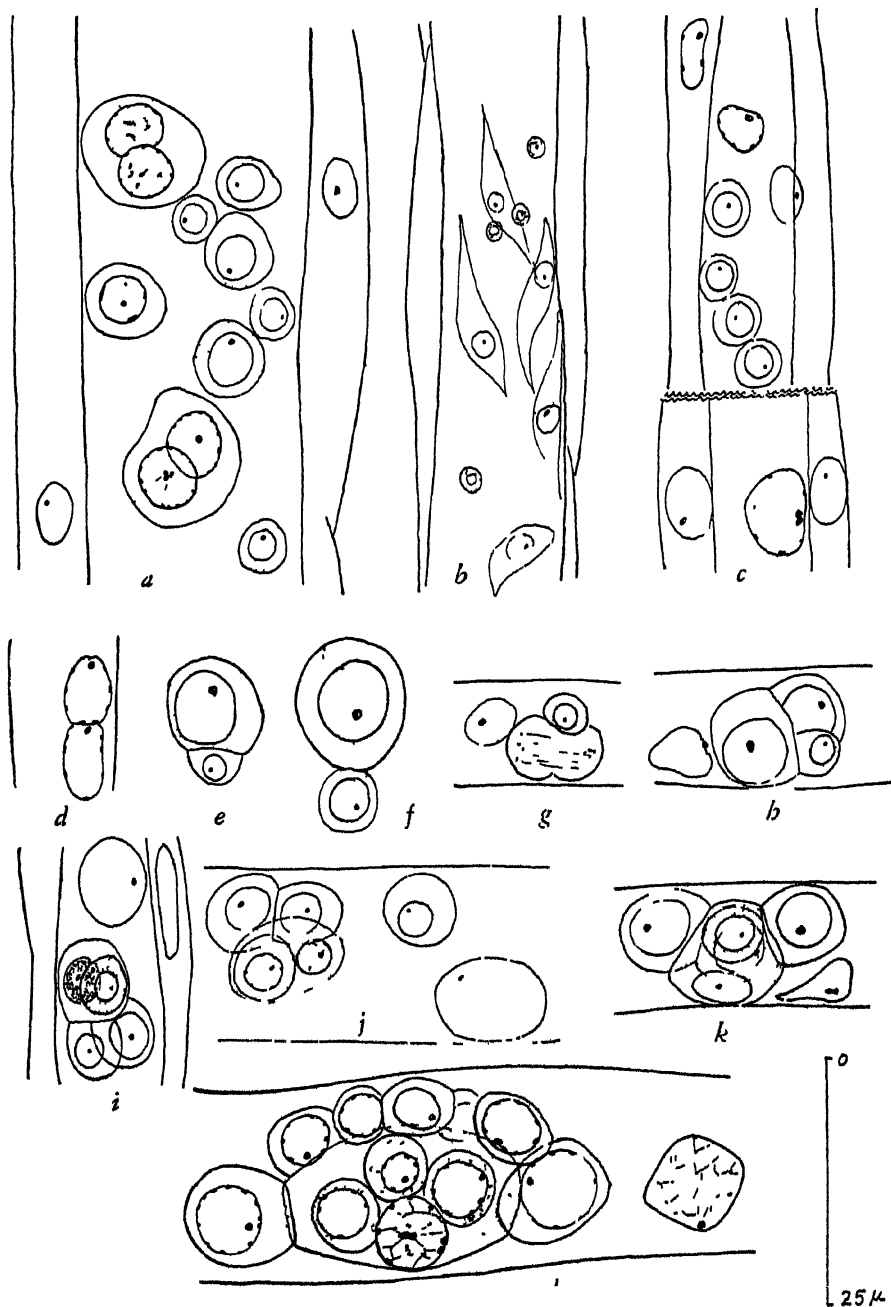


Fig. 7. a. Part of transitional *Dicyemonea brevicephala* showing two enlarged binucleate axoblasts. b. Part of early transitional *D. granularis* with both normal and enlarged fusiform axoblasts. c. Two parts of the trunk of a young *Dicyema balamuthi* containing two free nuclei, presumably derived from a single original axial nucleus by amitotic division. d. Part of the axial cell of a young *D. balamuthi* in which the axial nucleus is undergoing amitotic division. e. Elimination of a small cell, the future paranucleus, from the mother cell of an infusorigen in a transitional individual of *Dicyemonea californica*. f. Slightly later stage of the same. g. Very young in-

their correlation with the number of infusorigens being perfectly clear. In these species, in transitional individuals whose axial cells are still very crowded with axoblasts and vermiform embryos, the earliest infusorigens, which resemble young vermiform embryos in many respects, can often be most easily spotted by searching carefully in the vicinity of any small paranuclei that may be present.

The significance of the paranucleus will be discussed below as part of the whole question of the role of the infusorigen.

THE INFUSORIGEN

EMBRYOLOGY OF THE INFUSORIGEN

(Fig. 7, c-l)

The first cleavage of the mother cell of an infusorigen, following the elimination of the paranucleus, is unequal. The smaller cell is the forerunner of all the peripheral cells of the infusorigen, which later become egg cells; the larger one gives rise to the axial cell of the infusorigen and the germ cells contained therein.

Contrary to the early vermiform embryo, there is no period of delay before the next division of the larger cell, which is also unequal—the second cleavage of each blastomere occurring at nearly the same time. The young infusorigen then consists of four cells: a large cell that represents its axial cell and undergoes no further division, a small cell that promptly invades the axial cell and from which all the male germ cells of the infusorigen are derived, and two peripheral cells that remain outside the axial cell and are the forerunners of all the egg cells produced by the infusorigen. In some instances it appears that the division producing the small cell destined to invade the axial cell of the infusorigen precedes that which separates the definitive axial cell from the first of the peripheral cells.

There is no elimination of small chromatin granules during the very early development of the young infusorigen as is characteristic of early vermiform embryos.

Both the outer cells and the internal germ cells continue to multiply for a time by ordinary mitosis until the infusorigen consists of a large axial cell containing several germ cells similar to axoblasts in appearance and a number of peripheral cells partly enveloping the axial cell. The envelopment of the axial cell is usually incomplete to an advanced stage, so that in optical section the axial cell of a young infusorigen appears to be surrounded on only three sides by the peripheral cells, the side nearest the axial cell wall of the parent rhombogen usually being exposed. This is in marked contrast to the early vermiform larvae, in which the axial cell is very early enveloped completely by the peripheral cells.

In older mature infusorigens the axial cell appears fully enveloped. There is no differentiation of the body of the infusorigen into calotte and trunk. Instead, from this point on the cells undergo an entirely different course of development.

close by. *h*. Very young infusorigen of *Dicyema adsoita* showing definitive axial cell of the infusorigen. The first peripheral cell of the infusorigen, the first internal germ cell of the infusorigen (still outside the axial cell) and the paranucleus. *i, j*. Young infusorigens of *D. abilis*. The first internal germ cell has invaded the axial cell and the first peripheral germ cell has divided, forming a four-cell stage. The paranucleus lies close by. *k*. Similar young infusorigen in which one of the two peripheral cells has redivided, giving a five-cell stage. *l*. Optical section of a typical young infusorigen of *D. apollyoni* just before the onset of meiotic divisions. The axial cell is not entirely enveloped by peripheral cells. The paranucleus lies nearby in the axial cell of the

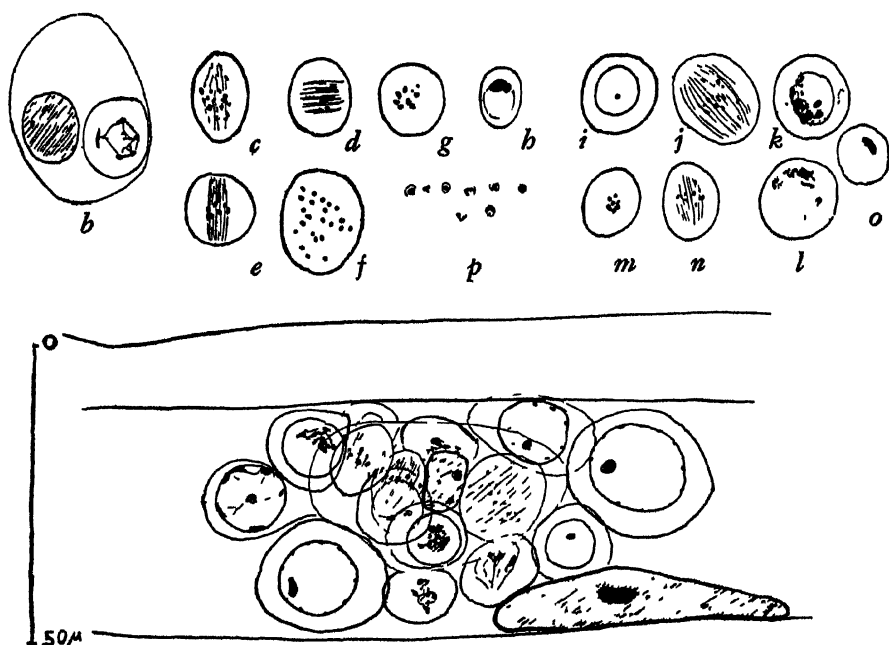


Fig. 8. *a*. Young infusorigen of *Dicyemenna californica* at time of onset of meiotic divisions of some of the cells. Characteristic clumping of the chromatin at one side of the nucleus, on strands of lighter staining material. In cells inside the infusorigen this is followed by reduction divisions leading to sperm formation. In the peripheral cells it is followed by a swelling of the cell as a whole and its nucleus. The reduction divisions of the egg do not occur until after sperm penetration. The paranucleus lies near by. Comparison with the paranuclei of a younger infusorigen shows a progressive increase in size until it becomes very similar in size and appearance to the axial cell nucleus of the parent rhombogen. *b-p*. Internal or male germ cells of the infusorigen: *b*. *Dicyema balamuthi*, axial cell of an infusorigen and one of its contained germ cells, showing chromatin clumped on light-staining network. *c*. *Dicyemenna bicephala*, first meiotic division of one of the germ cells. *d*, *e*. *Dicyema apollyoni*, second meiotic division of internal germ cells of an infusorigen. Seven or eight dyads with the appearance of single chromosomes are present on the metaphase plate. *f*. *Dicyemenna californica*, polar view of metaphase plate of spermatogonial division. At least twenty-three chromosomes can be seen. *g*. *D. californica*, polar view of male cell in second meiotic division. Eleven or twelve chromosomes can be made out. *h*. *Dicyema apollyoni*, spermatid. *i-p*. Male cells in various stages from a single infusorigen in a transitional individual of *Dicyemenna californica*: *i*. Spermatogonium before any changes preceding meiosis. *j*. Spermatogonial division. *k*, *l*. Clumping of chromatin at one side of nucleus preceding meiosis. *m*, *n*. Polar and side views of secondary spermatocyte division showing approximately eight dyads on the metaphase plate. The chromosomes and cells of dicyemids are so small that tetrads and dyads could not be distinguished as such, but appeared as just slightly larger chromosomes than those of other stages. *o*. Spermatid. *p*. Group of sperm cells.

MATURATION OF GERM CELLS AND FERTILIZATION

(Figs. 8 and 9)

Some of the axoblast-like cells inside the axial cell of the infusorigen continue to multiply by ordinary mitotic divisions. Rather early, however, the nucleus in others swells up noticeably; its chromatin is gathered near one pole in strands or as small knots of chromatin on strands of lighter staining material, forming a sort of radiating anastomosing network of fibrils. The chromosomes become individualized and the cell undergoes two successive divisions, resulting in smaller cells each

time. The smallness and crowding of the chromosomes has made it difficult to get an accurate count of their number but estimates on the clearest cases indicate a reduction in chromosome number by about half during these divisions. After the second division the chromosomes become lumped together in a tight little knot. The cytoplasm is almost all sloughed off and quickly disintegrates. The small tail-less sperm lie scattered about near the periphery of the infusorigen and attach

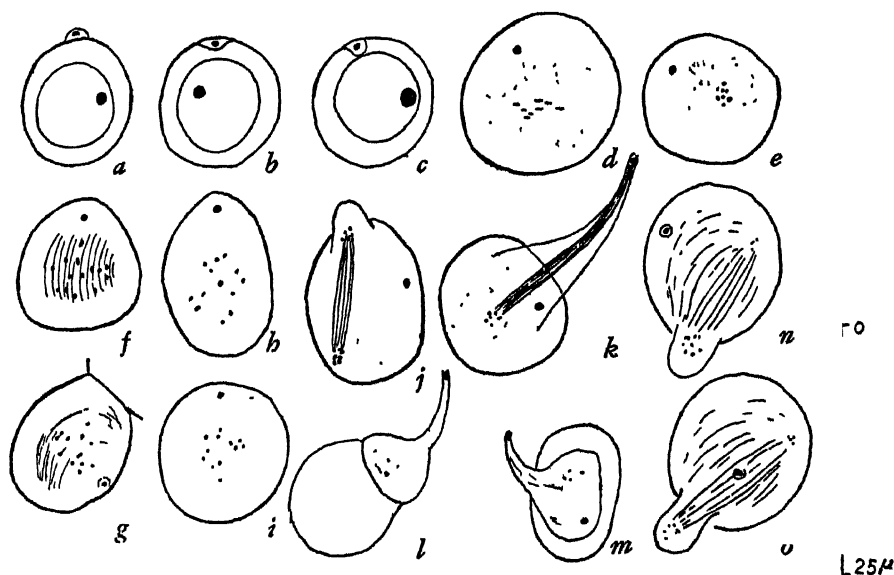


Fig. 9. Fertilization and polar body formation of the egg cells. *a-c.* *Dicyemenna adscita*, three stages in the penetration of egg cells by sperm. *d, e.* *D. adscita*, shortly after sperm penetration. The germinal vesicle has broken down and the chromosomes are becoming aligned on a forming spindle—lateral and polar views. *f-i.* *Dicyema apollyoni*, egg cells showing chromosomes, approximately fourteen in number scattered on the spindle in early anaphase preceding the elimination of the first polar body. *j, k.* *D. apollyoni*, elimination of first polar body. Seven chromosomes could be seen in the group remaining in the egg cell. *l, m.* *D. balaenuth*, extrusion of first polar body. In this species the part of the egg containing the chromosomes and spindle appears sharply set apart from the remainder of the cytoplasm during the division. *n, o.* *D. balaenuth*, extrusion of the second polar body. Each group of chromosomes can be seen to consist of approximately eight or nine chromosomes.

themselves to the mature oögonia at or near the point where these are attached to the infusorigen.

Meanwhile the larger peripheral cells or oögonia have multiplied. Some of them show similar phenomena to the spermatogonia, with a clumping of the chromatin in small knots and strands at one side of the nucleus followed by a marked swelling of both the whole cell and its nucleus. The nucleus possesses a large nucleolus and small granules of chromatin distributed on an achromatic network. It is at this time that the sperm attach themselves to the egg cells.

Shortly before or just after the attachment of the sperm cell, the egg cell detaches itself from the infusorigen and lies free in the axial cell of the parent rhombogen. The sperm penetrates a short distance into the cytoplasm and remains there unaltered while the nucleus of the egg cell loses its membrane, forms a division spindle, and undergoes two rapid successive divisions with the elimination

of a polar body from one end of the cell each time. The spindles in these divisions are usually very long, with the two groups of chromosomes at opposite poles of the cell. The first division is generally rapid, the polar body in some species pushing out onto a long stalk-like process. The second division is more frequently found.

In some examples definite reduction by half of the chromosome number has been established, though the smallness of the chromosomes makes exact counts difficult even in the large egg cells. However, a few clear cut cases of reduction to half, plus a number of estimates of approximate number showing a reduction by approximately half, clearly indicate that these actually are meiotic divisions.

After the elimination of the second polar body, both the egg nuclear elements and the sperm reconstitute separate nuclei so that the egg has two pronuclei—the usual male and female pronuclei. During the early stages of reconstitution of the female pronucleus several nucleus-like vesicles are often formed around separate chromosomes or groups of chromosomes so that the egg cell may temporarily appear to have several nuclei of various sizes. These unite quickly, however, and it is far more usual to find eggs with two well-formed pronuclei than the earlier stages with several vesicles. Caullery and Lavalley (1912) found the same condition of early formation of several vesicles by the female nuclear elements during the reconstitution of the female pronucleus in the orthonectids.

The fertilized egg promptly begins to undergo cleavage divisions, leading to the formation of the infusoriform. As more egg cells continue to be freed from the infusorigen the first ones are displaced farther and farther from the infusorigen in the axial cell of the parent rhombogen. Thus there often results from each infusorigen present, a string of embryos with fully formed infusoriforms at the ends. These extend in either direction along the axial cell of the rhombogen.

In infrequent cases the first egg cells ripen and detach from the infusorigen before any sperm are ripe. These either degenerate or produce a compact half-sized embryo that seldom reaches full development.

The infusorigen is not capable of indefinite production of sperm and egg cells but after a certain time appears exhausted. It consists then chiefly of its axial cell, with perhaps a few germ cells still contained in it, a few egg cells attached to it, and numerous sperm scattered about it. Degenerative phenomena and disaggregation as described by Gersch (1938) may be evident during the final stages in the life of the infusorigen. Eventually only its axial nucleus is left lying free in the axial cell of the parent rhombogen, or by now secondary nematogen, where it constitutes the residual nucleus of Whitman (1883).

This account of the infusorigen agrees most closely with those given by Lameere (1916) and by Nouvel (1933, 1947). It differs in all essential points from the account by Gersch (1938) and in several respects from the earlier accounts by van Beneden (1876), Keppin (1892), Whitman (1883), Wheeler (1897), and Hartmann (1904).

THE INFUSORIFORM

(Fig. 10, *a-l*)

From a morphological standpoint the infusoriform is the most complicated stage in the known part of the life cycle of dicyemids. At first glance it appears to bear little resemblance to any of the vermiform stages.

Most earlier writers—van Benedon (1876), Wheeler (1897), Keppin (1892), Hartmann (1906)—believed the infusoriform the male, and some of them—Keppin, Hartmann—even pictured tailed sperm in it. Later authors, Lameere (1918), Nouvel (1933), Gersch (1938) consider it simply a form serving to disseminate the species in a manner analogous to that of a trematode miracidium. The most complete account of the infusoriform has been presented by Nouvel (1948).

To bring out certain details not emphasized in previous accounts and to facilitate discussion of the differences between various accounts of the infusoriform, the morphology of the fully developed infusoriform of *Dicyemeneea adscita* will first be presented in detail. The variation between species, the embryology, and the probable role in the life cycle will then be discussed.

MORPHOLOGY OF THE INFUSORIFORM OF *Dicyemeneea adscita*

The mature infusoriform is a small bilaterally symmetrical, nearly oval organism about 32μ – 36μ long, 26μ – 28μ broad at the widest part of the body, and 24μ – 25μ deep. It is broadest anteriorly, tapering slightly to a bluntly rounded posterior end.

The anterior end is smooth and chiefly occupied by two large refringent bodies situated in the two anterior-dorsal somatic cells, distending and completely filling them. The posterior part of the body is clothed with long cilia directed backward most of the time.

Near the middle of the body and close to the ventral surface is a group of four cells constituting the contents of the urn. The cytoplasm of these cells stains more densely than that of the other cells of the body. Each of them contains two nuclei plus an enclosed germ cell, similar to an axoblast though much smaller. The cells are arranged to form a sort of hollow cup, open in an antero-ventral direction. The space thus formed communicates directly with the exterior through a small pore in the ventral body wall of the infusoriform. The lateral lips of this pore are slightly raised and can be seen in the living organism when viewed directly from behind or in front.

Enveloping the contents of the urn on all but the open side are two large crescentic capsule cells forming the urn itself. Their nuclei, the largest present in the infusoriform, are located above the cells of the urn contents near their posterior margin, one on either side of the midline. These cells elaborate rounded proteinaceous granules, highly refractile in life, which line their inner margins immediately adjacent to the cells within the urn, forming a sort of jacket or basket-like envelope around them. These also open antero-ventrally toward the ventral pore.

Immediately above and slightly forward from the capsule cells, just below the dorsal ciliated cells are two large flattened cells that have heretofore almost entirely escaped notice. They are shown partly for the first time in the illustrations by McConnaughey (1941) of the infusoriforms of *D. californica* and *D. brevicephala*, but no comment was offered regarding them in that paper. Nouvel (1948) reported their presence, terming them the internal dorsal cells but saying nothing more about them. Their distinctiveness and special characteristics make them of some interest. In ordinary preparations these cells appear as broad, flattened,

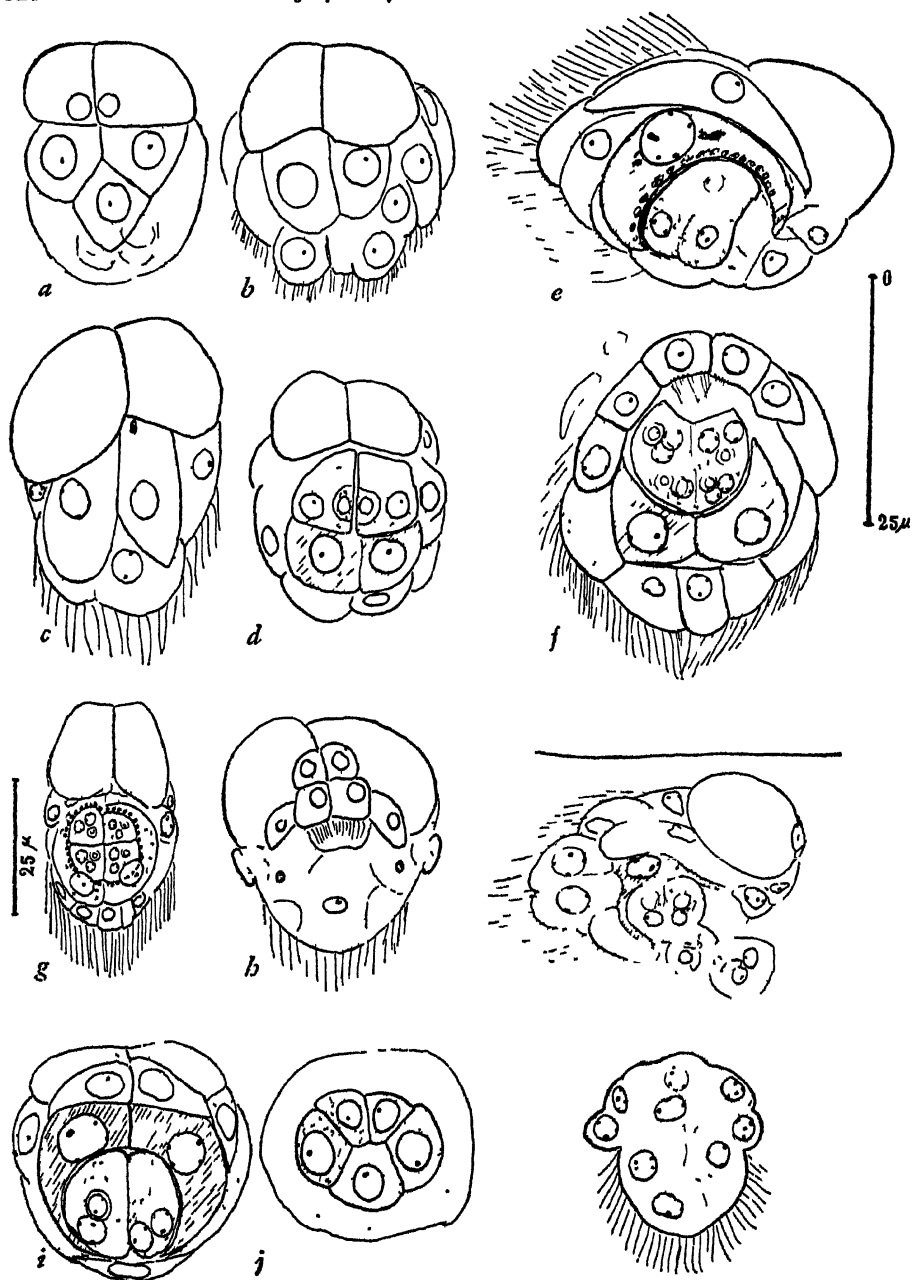


Fig. 10. Morphology of the infusoriform larva. *a. Dicyemenea granulata*, young infusoriform, dorsal surface, showing upper part of the refringent bodies, the nuclei of the cells containing them, and three dorsal ciliated cells. *b.* Optical section of the same individual at slightly lower level showing the two dorsal internal glycogen cells. *c. D. adscita*, optical frontal section of very young infusoriform at level of the dorsal internal glycogen cells. *d.* *Dicyema acciaccatum*, frontal optical section of very young infusoriform at the level of the urn, showing the invasion of two of the axial-type cells within the urn by the corresponding germ cells. The nucleus of the urn cell on either side has not yet divided to form the two nuclei characteristic germ cells. The nucleus of the urn cell on either side has not yet divided to form the two nuclei characteristic of the mature larva.

nonstaining cells, tilted downward and converging anteriorly between the posterior medial borders of the refringent bodies. Posteriorly they diverge somewhat and extend about three-fourths of the body length. These cells are not stained by ordinary histological stains but appear as clear spaces in the organism. Their nuclei are slightly irregular in shape and degenerate in appearance compared with those of the ciliated epithelial cells.

These two cells were found to store glycogen. Contrary to the results of Nouvel (1929, 1930, 1933), who reported glycogen stored in the ciliated epithelial cells and made no mention of these two special cells in these papers, I find in the species tested, that these two cells alone give a strong positive reaction either with the iodine test for glycogen or with Best's carmine, though traces can sometimes be found in other cells. They are completely negative for the aldehyde test for tryptophane, which gives a positive result for the refractile granules in the capsule cells.

The remaining cells, constituting the majority of the epithelial cells, are divisible into two groups. The ciliated cells cover the dorsal, lateral, posterior, and ventral parts of the body behind the refringent bodies and behind the ventral pore (17 cells). An anterior group of eight smaller lighter staining cells is situated ventrally, beneath the refringent bodies and anterior and lateral to the ventral pore. Four of these, situated immediately in front of the cavity of the urn, are larger than the other four that extend in a posterior dorso-lateral direction from them, two on each side. Two of the first four of these cells form the anterior margin of the cavity of the urn, and from these two cells cilia extend into the cavity of the urn. It is these cilia with their basal granules that were evidently mistaken for sperm by Keppin and Hartmann, as Nouvel (1933) has pointed out.

Lameere considered the eight antero-ventral cells as homologous with the calotte of the vermiform individuals, the cells bearing the refringent bodies as corresponding to the parapolar cells, the rest of the epithelial cells as equivalent to the somatic cells of the trunk, and the urn capsule cells together with the cells within the urn as corresponding to the axial cell and its contained germ cells.

This leaves unaccounted for the two dorsal internal or glycogen cells that were unknown to him and that seem to have no differentiated homolog in the vermiform individuals. Also it seems evident that the two capsular cells of the urn are somatic in character rather than axial. The four cells contained within the urn, each with

e. Dicyemenea adscita, parasagittal slightly tilted optical section of a mature infusoriform larva showing the glycogen cell, the capsule cell, a part of the urn contents and the cell bearing the internal cilia of the urn cavity of one side. *f. D. abichi*, oblique frontal section of mature infusoriform passing anteriorly below the refringent bodies through the two cells bearing the internal cilia of the urn cavity, and posteriorly dorsally through the contents of the urn, the capsule cells and just below the posterior ends of the glycogen cells. *g. D. californica*, frontal optical section of mature infusoriform at the level of the urn. *h. D. granularis*, mature infusoriform showing disposition of cells on the ventral surface. *i. D. adscita*, transverse optical section of infusoriform. Top to bottom: dorsal ciliated cells; dorsal internal glycogen cells; capsule cells; part of contents of urn; ventral somatic cell. Other somatic ciliated cells lateral in position. *j. Posterior end of same infusoriform showing in i*, showing the five caudal cells. *k. Dicyema balamuthi*, parasagittal optical section through an infusoriform in the act of discharging the contents of the urn into the axial cell of the parent rhombogen. *l. D. balamuthi*, abnormal infusoriform of reduced size in which the refringent bodies, the urn and other structures, failed to differentiate. Such larvae are infrequent but when found are usually farthest away from the parent infusorigen in the row of embryos—that is, are the first formed larva. It seems probable that occasionally an egg cell detaches from the infusorigen before the sperm are ripe and produces an abortive embryo.

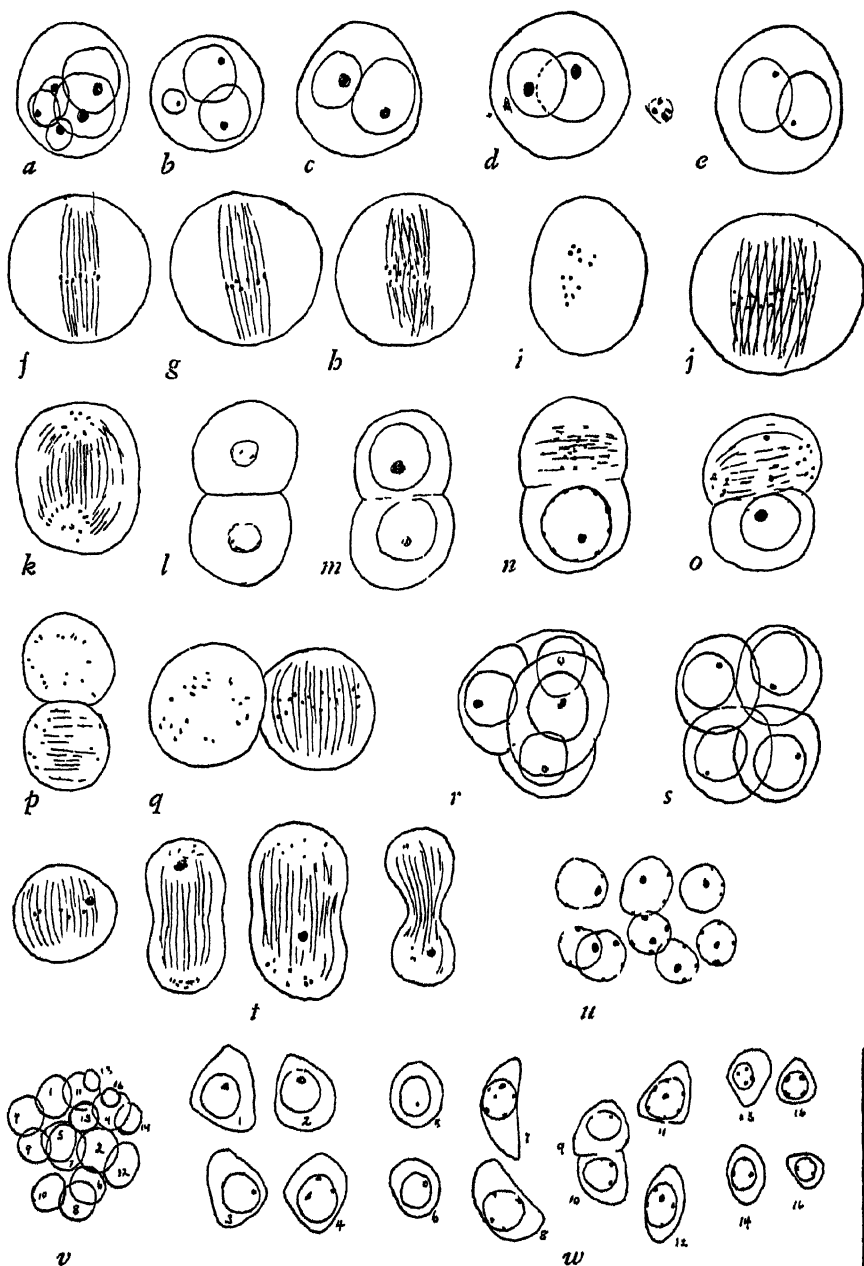


Fig. 11. Embryology of the infusoriform larva. *a, b. Dictyemmennea granularis*, fertilized egg cells following elimination of the polar bodies, showing early stages of the reconstitution of the pronuclei. *c, d. D. adaeita*, fertilized egg cells showing the male and female pronuclei. In *d* the polar bodies are also shown, one lying beneath, the other to one side of the egg cell. *e. Dictyema apollyoni*, fertilized egg cell with pronuclei. *f, g, h. D. apollyoni*, three views of the same egg cell at metaphase of the first cleavage, showing two spindles each with apparently seven chromosomes: *f*. High focal level. *g*. Lower focal level without change in orientation. *h*. Both views combined. *i*. Polar view of similar egg cell at metaphase of first cleavage. One of the chromosome groups

its two nuclei and inclosed germ cell, apparently do correspond to axial cells. The capsule cells might perhaps be considered modified uropolar cells.

Lameere's contention that the structure of the infusoriform represents a highly modified but recognizable vermiform individual seems to have good basis in fact. In any event it clarifies the discussion of the infusoriform and reduces it to a grade of organization coordinate with that of other stages in the life cycle.

VARIATION BETWEEN SPECIES

The infusoriforms of different species of dicyemids are remarkably uniform in structure. There is some variation in the size attained. Some are almost evenly rounded, others taper to a conical point behind. The cell number may vary slightly but this has not been established with certainty. A few species have only one nucleus in addition to the germ cell in each cell within the urn. Some species apparently void the contents of the urn more readily than others. The aspect of the nuclei, their size, the clumping of chromatin, staining characteristics of the cytoplasm, and the size of the refringent bodies also differ in some degree between various species.

EMBRYOLOGY OF THE INFUSORIFORM

The male and female pronuclei of the fertilized egg do not fuse before the first cleavage but each breaks down and resolves itself into a group of chromosomes that become arranged on separate spindles. Sometimes it is possible to distinguish readily the two groups of chromosomes at the metaphase and to observe that the spindles are separate or even oriented in a slightly different direction (fig. 11, *f-j*).

The first cleavage results in two cells of equal size with no readily distinguishable differences between them.

In the second cleavage the two cells divide in an orientation at right angles to each other so that often one of the blastomeres is observed from an equatorial, the other from a polar point of view. The four resulting cells are of equal size and not yet differentiated into recognizable classes. In some cases at least it is seen that during the telophase one or two larger pieces of chromatin lag behind on the spindle of one of the pairs of blastomeres, but not the other pair. These are eliminated as small vesicles containing chromatin, similar to those eliminated by young vermiform embryos.

appears to have seven, the other eight, chromosomes, though the eighth is so minute as to be uncertain. *j. Dicyemella brucephala*, metaphase of first cleavage showing two spindles in slightly different orientation. *k, l, m. D. adscita*, final stages of first cleavage and formation of two celled embryo. *n. Dicyema apolloni*, two-celled embryo, one of the cells entering upon the second cleavage division. *o. Dicyemella adscita*, two-celled embryo, one cell in late anaphase of second cleavage. Two pieces of residual chromatin can be seen lagging behind on the spindle. *p. D. granularis*, two celled embryo, both cells undergoing second cleavage division. *q. D. californica*, similar stage. *r, s. D. adscita*, four-celled embryos. *t. D. californica*, selected blastomeres from embryos during the second or third cleavage showing residual chromatin that lags behind on the spindle and passes to one of the resulting cells, later eliminated from the developing embryo. *u. D. adscita*, nuclei of an eight-celled embryo showing differentiation into four large and four slightly smaller cells. *v. D. adscita*, nuclei of a sixteen-celled embryo. *w.* The individual cells of the same embryo drawn separately to show cellular differentiation attained at the sixteen-cell stage. The four small cells (13-16) are the future germ cells each of which later invades one of the four larger cells (9-12) making up the contents of the urn. The two crescentic cells (7, 8) are the capsule cells of the urn. The other cells are not definitely identifiable at this stage. It appears that two of them († 5, 6) may be the two dorsal internal glycogen cells. The remainder probably represent the ordinary somatic cells.

The third cleavage results in an eight-celled morula of which four cells are just slightly smaller than the other four.

In the sixteen-cell stage (fig. 11, *v-w*) we find the first obvious and clear-cut differentiation. Here four small cells—the future germ cells—have been cut off internally from four slightly larger ones which they later invade, and which are the future contents of the urn. There are also at this time visible differences among the somatic cells. Two larger crescentic ones represent the capsule cells of the urn. Another pair probably are the two glycogen cells.

The four germ cells undergo no further division. The four cells representing the contents of the urn remain quiescent until they have been invaded by their respective germ cells. Then each undergoes one mitotic nuclear division not followed by a cytoplasmic division, which results in the definitive condition found in the fully formed infusoriform. A few species are reported to have only one nucleus in addition to the germ cell. In these this second division has evidently been repressed.

Meantime the other somatic cells have continued to divide, though not all at the same rate. By the time the embryo has attained the thirty-two-nucleus (28-cell) stage the anterior ventral group of cells in front of the urn and below the refringent bodies are set apart from the other somatic cells and the two refringent bodies are beginning to form. They are first evident as tiny pinhead-size clear vesicles in the cytoplasm of the anterior dorsal pair of somatic cells. They enlarge progressively till they distend the cell, occupying its whole volume and pushing the nucleus up against the cell wall.

Around the refringent bodies, especially on their ventral, medial, and posterior surfaces, are deposited small globules of another refractile substance, giving the surface a pebbled appearance similar to that of the sheath of proteinaceous granules surrounding the contents of the urn on all but one side. These granules may be the source of the secretion liberated by the infusoriform just before its escape from the parent rhombogen. They can be seen most easily in the living infusoriform.

Nouvel (1947) described for *Dicyma schulzianum* and *Dicymennea lameerei*, and implied for other species, a very unusual course of events in the fertilization of the egg cells.

According to his account there is only one division resulting in polar body expulsion. This takes place after the penetration of the egg cell by the sperm. The chromosome number in the egg nucleus is still diploid. This is followed by the expulsion of the sperm. The egg nucleus may then undergo a division without giving off a polar body. The two nuclei thus formed reunite, forming a single diploid nucleus entirely derived from the original egg nucleus. The cell then proceeds to undergo cleavage and to form the infusoriform larva.

This is at variance with all earlier accounts, though there had been no agreement among former writers as to what takes place at this time. It is also contrary to my own observations.

Polar body emission is correctly described by Nouvel, but this is the second polar body that is formed. Formation of the first one, which happens while the egg cell is still in contact with, or very close to the infusorigen, is rapid and difficult to detect.

I have found no evidence that the sperm is expelled from the egg before the first cleavage. It is possible that the small sperm-like body found adherent to early embryonic infusoriforms is the product of chromatin elimination similar to that taking place in young vermiform embryos. In the case of the infusoriform the eliminated chromatin tends to adhere more closely to the developing embryo than in the vermiform larva. Sometimes an additional sperm cell is found attached to the surface of a developing embryo. This could well account for such occasional appearances as shown in figure 51 D of Nouvel (1947).

ESCAPE AND BEHAVIOR OF THE INFUSORIFORM

When fully developed, the infusoriform becomes active while still inside the axial cell of the parent rhombogen. Its activity consists primarily in turning over and over in the same place, though it may sometimes push its way for some distance along the axial cell. Before its escape the infusoriform liberates a secretion from around the refringent bodies that causes the formation of a large vacuole around it. This vacuole grows progressively till the body of the rhombogen is distended at this point and the epithelium very thin and stretched out. The infusoriform acts as though partly paralyzed. With its cilia held rigidly in a reversed direction, it is gradually squeezed out backward, together with the contents of the vacuole, through the side of the rhombogen which immediately closes up, leaving no scar or any visible trace of the point of rupture. The liberated infusoriform sometimes swims away almost at once, sometimes remains nearly motionless with its cilia held rigidly in a reversed direction for one or two minutes before swimming off.

Once active, the infusoriforms are able to swim very rapidly. They usually go at once to the bottom of any vessel in which they are put and swim about over the bottom. When uninterrupted they may go an inch or more in a straight line, rotating on their long axes as they swim. If an infected cephalopod containing rhombogens is kept in a jar of sea water for several hours a large number of infusoriforms can usually be recovered by placing the water in a large separatory funnel and drawing them off at the bottom a few minutes later.

When placed in a small vessel of sea water or diluted octopus urine under a dissecting microscope, the living infusoriforms showed no apparent interest in other stages of dicyemids introduced into the water with them, no interest in newly hatched young octopus or squid, nor in any of the other animals so far tested, though their small size renders it difficult to tell in most cases. Surface scrapings or extracts of various intertidal animals, especially bottom forms, introduced among active infusoriforms in the ends of capillary tubes brought no noticeable response. After twelve hours or more in sea water some of them will usually be found to have voided the contents of the urn or to be undergoing general disaggregation. As a rule they do not live more than three or four days in small aquaria with aerated water. Specimens fixed after several hours of swimming about in sea water showed no significant changes in their structure, contrary to statements by Gersch and others.

When a number of living infusoriforms are observed on a slide under the microscope it is not unusual to see one of them swim about rapidly in an agitated manner, suddenly pause or move in a small circle and discharge from the posterior end a

large number of sperm-like bodies. These continue to squirm about independently for a time. They correspond closely to the sperm figured by Keppin (1892) and Hartmann (1907). The infusoriform may swim on, and in a short period, discharge two or three groups of these sperm-like bodies.

This observation at first seemed to support the contention that the infusoriform is the male, but careful study of the process showed that it is actually only the first step in the disaggregation of the individual. The sperm-like bodies are cilia with their basal granules, which come off in considerable numbers and sometimes leave the infusoriform almost bare or with irregular patches of cilia. The infusoriform itself usually undergoes general disaggregation within ten to fifteen minutes after it begins to lose cilia. The process occurs most frequently when the preparation has begun to lose water by evaporation so that the concentration of salts is increased.

THE SECONDARY NEMATOGEN

Occasionally a cephalopod is found in which some of the diacyemids show the secondary characteristics of rhombogens (accessory nuclei in the somatic and axial cells) but are giving rise to a generation of vermiform larvae. Often there are even one or more mature infusorigens present, with egg cells and infusoriform embryos to an advanced morula stage. These morulae then appear to disaggregate instead of completing their development. Usually a large number of axoblasts are present.

There has been little agreement about the phenomena leading to the resumption of nematogenic activity by rhombogens, and Gersch (1938) has even denied that it occurs at all.

That it does sometimes happen there can be no doubt. The writer has found cases that allow of no other interpretation, that is, individuals with advanced infusorigens and infusoriform embryos together with young vermiform embryos in the earliest stages of development. Several questions, however, need further clarification: 1. What is the source of the axoblasts from which the new generation of vermiform larvae arises? 2. Are these vermiform larvae essentially different from those produced by primary nematogens? 3. Is the phenomenon a regular and necessary part in the life cycle or something that happens under special conditions and perhaps only in certain species?

Whitman (1893) and Hartmann (1906) believed that the last germ cells produced by the infusorigen, instead of developing into egg cells and producing infusoriforms, simply divide to form axoblasts or develop directly into vermiform embryos. It was believed that these embryos were capable of tolerating sea water and migrating to other cephalopods where they start new infections and await fertilization by males (infusoriforms) which likewise were thought to migrate from one cephalopod to another.

Benham (1901), in Lankester's *Treatise on Zoology*, stated that the peripheral cells of the infusorigen form egg cells that become infusoriforms [after which] the germ cells within the axial cell of the infusorigen are liberated and give rise to vermiform larvae. This is probably a misreading of the literature by Benham, as he is not known to have studied the diacyemids directly.

Lameere (1916) and Nouvel (1933) believed that the cells of the developing infusoriform, upon its disaggregation, become axoblasts producing new vermiform larvae, which thus arise by a peculiar sort of polyembryony—the cells of one sort of highly specialized individual reverting, midway in its development, to the condition of primitive germ cells and giving rise to individuals of a very different sort. According to them the vermiform larvae so engendered do not migrate from one cephalopod to another but simply continue the infection in the same host in the same manner as the ordinary vermiform larvae. This view was also adopted in my brief review of the life cycle (1938).

After a restudy of the question I have arrived at a different interpretation which, however, cannot be said to be conclusively established. It is frequently found that in active rhombogens a few ordinary axoblasts still persist, apparently in good condition. It is thought that under certain conditions these are able to resume their activity and again begin producing more axoblasts and vermiform larvae in the usual manner. This would leave the infusorigen and its products out of the picture as far as the production of vermiform larvae is concerned.

During the period of transition from nematogen to rhombogen it is sometimes noticeable that the last vermiform larvae appear stunted and poorly developed. It would seem that there is a competition between the products of the two modes of reproduction for the available nutritive substances dissolved in the axial cell, that the production of infusoriforms is the more vigorous and suppresses the activity of the axoblasts through starvation and possibly also by some direct influence. At the time of exhaustion of the infusorigens, when their activity is reduced or stopped, it is thought that the situation may be reversed, and that any axoblasts that have survived and persisted through the period of rhombogenic reproduction may be able to resume their activity and produce one or more generations of vermiform larvae before the death of the old rhombogen. The resumption of the nematogenic mode of reproduction is viewed, not as a constant and invariable part of the life cycle but as a more or less accidental consequence of the persistence of some axoblasts in good condition through the rhombogen period in some of the individuals.

RÉSUMÉ OF THE LIFE CYCLE

The known part of the life cycle as interpreted in this paper is summarized in figure 12. A single arrow represents a new generation arising from germ cells within the preceding. A double arrow indicates merely the transformation of an individual from one phase to another. Parenthesis indicates that the form inclosed does not leave the axial cell of the individual in which it arose.

GENERAL CONSIDERATIONS

SPECIALIZATION WITHIN THE GROUP

The dicyemids show a number of peculiarities that can be interpreted as possibly pointing out the general directions in which specialization has proceeded within the group. It will be convenient to think of these in two categories: (a) primitive or generalized characteristics that appear during the embryological development of some or all species and that are more clearly retained in some than in others;

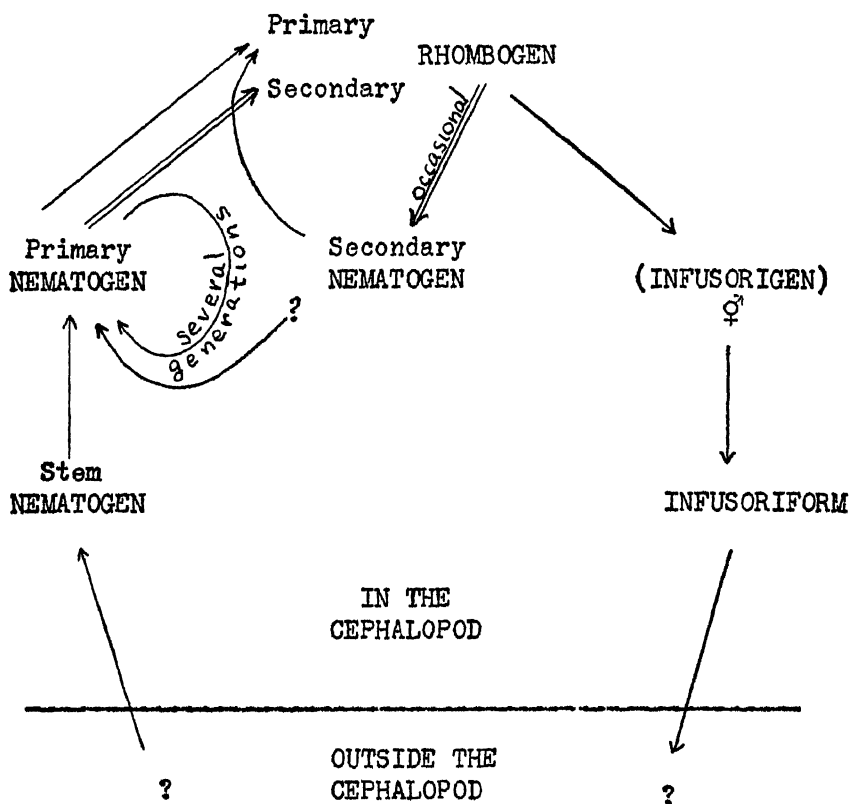


Fig. 12. Diagram of the life cycle as interpreted in this paper.

(b) terminal specializations, specialized conditions, or adaptations of particular species or groups of species.

These can be arranged in related pairs showing general trends from more generalized to more specialized conditions as follows:

GENERALIZED	SPECIALIZED
1. large, variable number of somatic cells.	reduced number of somatic cells, tendency to constant number.
2. medium to large size, prolific formation of embryos.	very small size, few embryos.
3. conical orthotopal calotte.	discoid or plagiostomal calotte.
4. axial cell not penetrating the calotte to the propolar cells.	axial cell penetrates forward to the propolar cells, often inflated and in extreme cases (heteroecyemid condition) branching into inflated cells of the calotte.
5. second axial cell present as small abortive cell in vermiform larvae.	no second axial cell present in the vermiform larvae.
6. axial and paraneuroni maintain their integrity.	additional free nuclei frequently present be-

- | | |
|--|--|
| <p>7. axial cell nucleus of inclosed vermiform larvae located between the first two axoblasts when larva attains full development.</p> <p>8. caudal somatic cells verruciform, other verruciform cells frequently present.</p> <p>9. infusoriform with two nuclei and one germ cell in each cell in the urn.</p> | <p>axial cell nucleus of inclosed vermiform larvae anterior to the first two axoblasts in developed larvae. Further multiplication of axoblasts may occur in larva before its escape from parent nematogen.</p> <p>caudal cells not verruciform, some species without any verruciform cells.</p> <p>infusoriform with one nucleus and one germ cell in each cell in the urn.</p> |
|--|--|

If we accept the foregoing criteria of what constitute generalized and specialized conditions within the group it becomes apparent that the genus *Dicyemeneea* rather than *Dicyema* is the more generalized and primitive.

Within each genus there are some species that seem to represent best the generalized generic type or model characteristics, and various species or small groups of species showing departures from this in certain directions.

Thus within genus *Dicyemeneea*, *D. californica*, *D. abelis*, *D. acscita*, and *D. granularis* seem to represent the generalized condition very well. *D. lanecei* and *D. abasi* show much reduction in size, loss of verruciform cells, and, in *D. lanecei*, a forward extension of the axial cell to the propolars. *D. brevicephala* and *D. abbreviata* show greater cell constancy, increasing flattening of the calotte, increasing disproportion in size between the pro- and metapolar cells, and increasing differentiation of the cephalic swelling leading to the condition in the heterocyemid *Conocyema adminicula*. *D. eledones* and *D. gracile* show unusually large size together with loss of verruciform cells and, in *D. gracile*, of the larval second axial cell. In them the axial cell extends forward to the propolars, being broadly rounded or even somewhat inflated within the calotte.

Genus *Dicyema* as a whole is characterized by one less metapolar cell with no second axial cell rudiment in the larvae. There is a tendency for the axial cell to extend forward into the calotte, swelling somewhat at the anterior end. Likewise it has a tendency toward irregularities in the free nuclei within the axial cell, caused by amitotic divisions of the axial cell nucleus or of some of the parauclei, together with formation of other free nuclei or nucleus-like bodies from some of the germ cells without direct relation to the formation of infusorigens.

Typical species—*D. microcephalum*, *D. clausianum*, *D. moschatum*, *D. macrocephalum*, *D. balamuthi*, *D. apollyoni*, *D. misakiense*, and *D. typus* are similar but with lower cell count, no verruciform cells, and slightly smaller average size. *D. orientale* and *D. schulzianum* show a tendency toward a flattening and telescoping of the cells of the calotte, reminding one of the condition in *Dicyemeneea brevicephala*. This is even more pronounced in *Dicyema truncatum*, which in addition shows a rotation of the position of the metapolar cells relative to that of the propolars. In this genus the three species in which the axial cell ends anteriorly at the posterior end of the metapolar cells—*D. monodi*, *D. acciaccatum*, and *D. acheroni*—are all small, without verruciform cells, and in general seem specialized and atypical rather than primitive and generalized. It seems possible that they may have arisen separately from the other species of the genus.

D. sullivanii is similar to the more typical members of the genus in size and cell number but does not exhibit the forward extension of the axial cell to the propolars

AXIAL CELL

It may be useful to bring together in one place a summary of observations and conjectures on the axial cell, its relation to the germ cells and to the reproductive cells of the orthonectids.

In the most generalized orthonectids the egg cells of the female form a compact mass filling the body and surrounded by a single layer of ciliated somatic cells. The sperm are developed in a discrete testis (fig. 13, *a*).

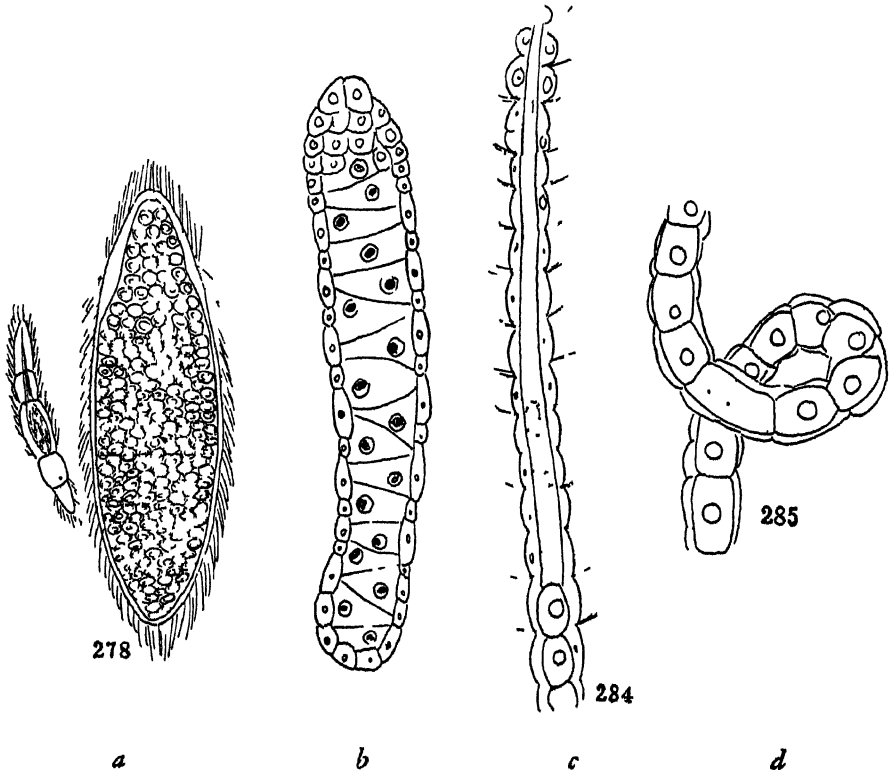


Fig. 13. Orthonectids. *a*. Typical orthonectid, *Rhopalura ophiocoma*, male discharging sperm near the genital pore of the female. From Lameere after Caullery and Lavalley. *b* *R. metchnikovi*, female, germ cells oriented in a double row. From Hartmann after Caullery and Mesnil. *c*. *Stoechartrum quadria*, anterior end. *d*. *S. quadria*, part of trunk showing egg cells in single linear series, one to each apparent segment. One of the segments has developed as a testis. From Caullery and Mesnil.

In some species the ova are aligned in one or two longitudinal rows (fig. 13, *b*).

Stoechartrum has a long filiform body with an appearance of segmentation, each division of the body containing one ovum. The organism is hermaphroditic. The segments constituting testes produce many spermatozoa (fig. 13, *c-d*).

In the stem nematogens of dicyemids there are three (two in one species) large axial cells in linear series. The young larvae show an additional cordon of several aborted axial cells anterior to the definitive ones.

In embryonic vermiform larvae of most *Dicyemennae* there are two axial cells, one abortive. The first germ cell or axoblast is formed by a complete cell division

of the large central cell of the young embryo so that there is formed a row of three internal cells surrounded by the somatic cells. The anterior cell, representing an abortive axial cell, degenerates; the large central cell becomes the definitive axial cell; the posterior cell constitutes the first axoblast and invades the axial cell.

In larval *Dicyema* the small abortive second axial cell is not usually formed; only the definitive axial cell and the first axoblast are found in the young larvae.

The early embryology of the infusorigen differs from that of a vermiform larva chiefly in the precocious development of the axial elements. Instead of remaining quiescent until somatic cells have largely surrounded the central cell, the differentiation of axial elements starts first and continues simultaneously with the early differentiation of the peripheral cells. The first distinctive act is the elimination of a small cell that becomes the paranucleus. This is here interpreted as equivalent to the first division of the central cell in larval *Dicyemennca* with the formation of an abortive second axial cell. In this case, the eliminated cell, not being inclosed in a developing embryo but lying free in the axial cell of the parent rhombogen, develops into a large nucleus-like body—the paranucleus of Whitman. The formation of the first internal germ cell proceeds as in vermiform larvae but much earlier, before the axial cell is surrounded by peripheral cells. The later fate of the peripheral and internal cells differs altogether from that of the corresponding cells in vermiform embryos.

In the infusoriform embryos the four axial, or urn, cells and four germ cells are set apart at the sixteen-cell stage. Each germ cell later invades the corresponding urn cell. In most species there is a single additional mitosis of each urn cell nucleus not followed by cytoplasmic division so that each of the four cells consists of a binucleated cell enclosing one germ cell. These four cells constitute the contents of the urn of the infusoriform.

Thus in all known stages of the dicyemids each axial cell and the corresponding first inclosed germ cell are at first coördinate cells derived from a mitotic division of a single large reproductive cell. Further, in those forms and stages in which more than one axial cell is represented, the additional axial cells appear to be derived by early division of a single original cell representing all the reproductive and axial elements in the embryo. This is certainly true in the case of larval primary nematogens of *Dicyemennca* and presumably true for larval stem nematogens.

It seems then that Lameere's concept that the axial cell is a primitive germ cell that has lost its reproductive capacity and developed into a large follicular cell for the protection and nourishment of the other germ cells and embryos developed from them, is correct.

The evidence indicates that the ancestral form for the dicyemids was probably a ciliated sexual vermiform organism with a linear series of inclosed reproductive cells. Among the orthonectids *Stoechartrum* fits this formula rather well and exhibits a grade of organization indicating a probably close relationship between this genus and the forms from which the dicyemids were derived.

The principal innovations in dicyemid organization are the inclosure of the active reproductive cells in one or a few large follicular cells, the axial cells, and the curious degradation of the sexual generation to a mere group of reproductive cells that never leaves the axial cell of the form giving rise to it.

A definitive conclusion regarding the phylogenetic relationships of the dicyemids must, however, await an elucidation of the unknown part of the life cycle between the infusoriform and the stem nematogen.

CAUSE OF THE CHANGE OF PHASE

The factors that bring about the change from the nematogen to the rhombogen phase are not clear. That the change occurs in most of the individuals present in a cephalopod at about the same time, and that young individuals liberated during this time or afterward apparently develop directly into rhombogens without previously functioning as nematogens, indicate that the change of phase is probably induced by some environmental agency affecting the whole population rather than being simply a maturing of the individual parasites.

The change does not appear seasonal in character, as populations of rhombogens are found in mature octopuses at all times of the year, whereas young octopuses taken at the same time harbor nematogens.

The fact that the change generally occurs at about the time the host reaches sexual maturity has suggested to several investigators that humoral changes in the host, incident to the attainment of sexual maturity, are responsible for the change in the parasites. This is a very plausible theory since in various other groups, humoral changes of animals have been shown to exert far-reaching effects upon their parasites, but as yet there is no experimental evidence in this instance.

The fact that occasional nematogens can usually be found by careful search even in populations predominantly rhombogen, and that certain clusters of individuals may be composed chiefly of nematogens whereas others in the same cephalopod are predominantly rhombogen show that, whatever the cause of the change, it is not uniformly and simultaneously operative over the entire population. This suggests that perhaps the parasites themselves may be at least partly responsible for the changes; that the great crowding of the parasite population with consequent alteration of conditions within the host kidney or urine, perhaps the liberation of metabolic products or hormones or the depletion of available nutrients by the parasites, induces the change. The fact that the change of phase is accompanied by numerous degenerative alterations, as amitotic division of the somatic nuclei and extensive degeneration and digestion of the axoblasts, lends some support to this hypothesis. Uneven population and crowding of the various kidney lobules might account for irregularity of the change of phase between different clusters of parasites in the same host.

SUMMARY

The known part of the life cycle of the dicyemids is essentially that presented by Lameere (1916, 1918, 1919).

The possible derivation of the dicyemids from *Stocchartrum*-like orthonectiform ancestors is discussed. Within the dicyemid group the genus *Dicyemonnea* rather than *Dicyema* appears to contain the more primitive generalized members. The heterocyemids are regarded as extreme modifications of the dicyemid form, possibly polyphyletic in origin.

The existence of stem nematogens and of secondary nematogens for some species has been confirmed. A simpler explanation of the origin of the axoblasts giving rise to vermiform larvae in secondary nematogens is suggested.

The origin and fate of the small degenerate cell just anterior to the axial cell in larval *Dicyemenna* is traced. It is interpreted as a rudimentary second axial cell and its possible phylogenetic significance discussed.

The chromatin granules eliminated by young vermiform and infusoriform embryos are interpreted in the light of the theory recently offered by Goldschmidt and Lin (1947) concerning chromatin diminution in cell-constant animals. It is shown that this elimination occurs only from the vermiform and infusoriform embryos, which develop into cell-constant stages, but not from embryonic infusorigens in which all the cells become reproductive cells.

The relationship of the paranuclei to the infusorigens is confirmed. The paranucleus itself is regarded as an aborted second axial cell of the infusorigen, morphologically equivalent to that formed by larval *Dicyemenna*.

The hermaphroditic character of the infusorigen is confirmed. The formation of sperm and egg cells by the infusorigen is confirmed. Chromosome reduction is demonstrated for one species and indicated though not proved for others. Fertilization and restoration of the diploid chromosome number is demonstrated. The sperm plays a normal role in the fertilization of the egg cell.

The morphology of the infusoriform is examined in greater detail than heretofore. It is considered a swarming form serving to disseminate the parasite, presumably to another host species, somewhat in the manner of a miracidium. The two dorsal internal cells are shown to store glycogen. A simplified method of obtaining living infusoriforms in large numbers for infection experiments was devised. All such experiments to date have given negative results.

Both male and female octopuses were found to shed infusoriforms at all times of the year without regard to the breeding season. They were not found to shed vermiform larvae.

The relationship of the Mesozoa as a whole to other groups of animals is still obscure, though it seems probable that they were derived from higher metazoa rather than forming an intermediate link between these and the protozoa, as several writers have already maintained.

The flatworms, the ciliurans, and the coelenterates have been suggested by various authors as probable ancestors of the Mesozoa. Perhaps the most acceptable opinion is that expressed by Stunkard (1937) who regards them as degenerate or highly specialized flatworms, derived, not from any existing group of the Turbellaria, but from a remote ancestor of all present flatworms, probably before the origin of the digenetic trematodes or cestodes.

Hyman (1940) has suggested that their structure may indicate that one of the earliest steps in metazoan organization was the relegation of the reproductive cells to the interior, rather than the formation of a hollow gastrula.

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STRUCTURE AND MORPHOGENESIS OF
TRICHOMONAS PROWAZEKI ALEXEIEFF
AND TRICHOMONAS BRUMPTI ALEXEIEFF

BY
BRONISLAW M. HONIGBERG

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STRUCTURE AND MORPHOGENESIS OF TRICHOMONAS PROWAZEKI ALEXEIEFF AND TRICHOMONAS BRUMPTI ALEXEIEFF

BY

BRONISLAW M. HONIGBERG

INTRODUCTION

SEVERAL accounts of *Trichomonas prowazeki* Alexeieff, 1909, from amphibians (Alexeieff, 1909; Koloid and Swezy, 1915; Escomel, 1925; Grassé, 1926), and at least one account of the species from reptiles (Parisi, 1910), are found in the literature. However, this paper deals with many structural and morphogenetic details which were not brought out in the previous studies. The same is true, to an even greater degree, of *Trichomonas brumpti* Alexeieff, 1912, for only a single, rather scanty account concerning the structure of this species has been given by Alexeieff (1912).

During the present study *T. prowazeki* was found in caudate and acaudate amphibians and in squamate reptiles. Although it is possible that important physiological differences may exist among the strains of the four-flagellate trichomonadids which are harbored by various amphibian and reptilian hosts, these differences are not reflected in the morphology of the symbiotes. Consequently, it seems necessary to consider all these flagellates as belonging in a single species. The other species, *T. brumpti*, appears to be restricted to chelonians.

I am grateful to Professor Harold Kirby, who supervised this research and was always ready with advice and encouragement. Credit is also due to Dr. Wade R. Fox, who gave me free access to the numerous reptiles that he caught in the field and identified. Messrs. S. Perkins and Charles Shaw of the San Diego Zoölogical Gardens were most helpful in supplying me with intestinal material from foreign reptiles.

MATERIALS AND METHODS

In the present investigation I observed *Trichomonas prowazeki* in the large intestine and rectum of various amphibians and reptiles. Among amphibians this trichomonad was found in the California newt, *Triturus torosus* (4 individuals) and in three species of North American anurans: the southern toad, *Bufo terrestris* (1 individual), the yellow-legged frog, *Rana boylei* (2 individuals), and the bullfrog, *Rana catesbeiana* (2 individuals). Flagellates morphologically indistinguishable from *T. prowazeki* were present in two species of geckos, *Gekko vittatus* (1 individual) and *Gehyra* sp. (2 individuals) from the Palau Islands, and in two species of garter snakes, the Pacific coast garter snake, *Thamnophis elegans atratus* (2 individuals), and the California coast red-sided garter snake, *Thamnophis ordinatus infernalis* (1 individual). Except for the three geckos, all the hosts were caught in the field and killed. Their intestinal contents were then thoroughly examined. The geckos were kept in the San Diego Zoölogical Gardens and only the fecal material and the rectal and cloacal fluids could be used for study. Although

The author of this paper is now at the University of Massachusetts.

Kofoid and Swezy (1915) described *T. prowazcki* from *Triturus torosus*, none of the other afore-mentioned host species has hitherto been found infected with this flagellate.

Strains of *T. prowazcki* from two bullfrogs, both geckos belonging to the genus *Gehyra*, one Pacific coast garter snake, and one California coast red-sided garter snake could be maintained in culture. The cultures derived from the intestinal material of the snakes were "pure" for *T. prowazcki*; those derived from the remaining hosts also contained *Tritrichomonas batrachorum*, which was scarce in the strains from the bullfrogs and quite abundant in those from the geckos.

Cultivated strains provided the primary source for study of the structural and morphogenetic details.

Trichomonas brumpti was encountered in the fecal material from three Galápagos tortoises, of which one belonged to the species *Testudo hoodensis* and the other two to an unidentified species of the same genus, as well as from two North American desert tortoises, *Gopherus agassizii*. All tortoises were kept in a single enclosure in the San Diego Zoological Gardens. Thus it is impossible to know whether all the host species would have harbored the flagellate under natural conditions. On the other hand, however, it is quite evident that the symbiote fails to exhibit strict host specificity.

A strain of *T. brumpti* from one of the two hosts belonging to *Testudo* sp. could be maintained in culture. Most data concerning the finer morphological details of the nondividing forms and all data pertaining to morphogenesis were derived from study of the preparations made from this culture.

Balamuth and Sandza's (1944) yolk infusion was found to be the most satisfactory culture medium for maintaining the flagellates. The medium was modified by the addition of sterile horse serum (1 part of serum to 20 parts of yolk infusion). The presence of the serum served a twofold purpose. It prolonged the life of the culture and facilitated the affixation of the culture material to the cover slips. Whole-egg slants covered with cold-blooded Ringer solution, to which sterile horse serum (1:20) and a loopful of rice starch were added, were sometimes employed.

The flagellates kept in culture were studied in dark field.

The permanent preparations were fixed in Hollande's cupric picroformol, Bouin's fluid, or Schaudinn's fluid.

Hollande's and Bouin's fluids were used routinely prior to impregnation with strong protein silver. They were also employed together with Schaudinn's fluid for fixing preparations which were to be stained with iron hematein (or hematoxylin).

The most important single factor in this work was Bodian's (1937) protargol technique. This technique, which was introduced by Cole and Day (1940) into the field of protozoölogy, permitted Kirby and Cleveland to achieve spectacular results within recent years. Although most useful if handled properly, it is also very capricious. Since the present investigation is largely based on the results obtained through the use of protargol impregnations, some discussion of the difficulties and remedies involved in their preparation seems to be in order.

The protargol technique consists of several steps. The first step requires the application of Mallory's bleach. In 1947 I described the basic procedure involved

in the use of this bleach for protozoan material. Since that time I have introduced many modifications in this procedure. The greatest difficulty in using the bleach was the loss of much, and often of all, of the material in the 5 per cent oxalic acid. This loss is particularly heavy in culture smears. I therefore started to reduce the strength of the acid as well as the time of exposure to that reagent. These reductions necessitated a parallel modification of the potassium permanganate solution. It may be mentioned here that the strength of the KMnO_4 in itself affects the loss of the material. Material taken from a strong solution of KMnO_4 into an oxalic acid solution of a given potency will be lost more readily than a similar preparation taken from a weaker KMnO_4 into an oxalic acid solution of the previously employed potency. After a long series of trials it was established that a three- to five-minute bath in 0.25 per cent KMnO_4 , followed by a thorough, but never vigorous, rinsing in distilled water, and a subsequent two- to four-minute bath in 1-2 per cent oxalic acid is satisfactory for almost any smear. The shorter times of exposure and the weaker solution of the oxalic acid have been used for the thin and fragile smears of the culture material. Little, if any, material is lost under these circumstances. In view of the properties of the presently available protargol, any attempt to impregnate without previous application of the bleach will invariably result in complete failure.

The second step involves the actual impregnation with activated strong protein silver. The postwar medicinal protargol was found to be entirely unsatisfactory for technical purposes. However, several lots of the technical protargol could be used with some degree of success. With fecal material, the procedure is fairly simple and does not differ in any way from that suggested by Kirby (1945). On the other hand, the culture material presents often an entirely different problem. Depending upon the amount of serum in the medium and the nature of the organism, the strength of the protargol solution and the time of impregnation may be varied. The type of procedure to give satisfactory results may be learned only through experimentation. It is generally true that the more serum present in the medium the heavier will be the impregnation. I found that sometimes smears exposed for four to six hours to a 0.5 per cent solution of activated protargol contained beautifully impregnated organisms, whereas on other occasions forty-eight hours of exposure to a 1 per cent solution had to be employed. Although usually the optimum temperature for impregnation is 37° C., at times better results may be obtained at room temperature.

The third and final step involves the development of the impregnated preparations. By varying the time of rinsing before the initial hydroquinone bath and also the time of the final bath in sodium thiosulfate, the results may be greatly modified. The longer the periods the less intense will be the impregnation. The duration of the intermediate rinsings in distilled water and of the baths in hydroquinone, gold chloride, and oxalic acid solutions remain standard and conform to the original instructions given by Kirby (1945).

At times a counterstain with fast green, by introducing a greater contrast between the cytoplasm and the impregnated structures, may be helpful in studying some of the organelles.

Thus it is obvious that the protargol technique has not as yet been, and probably

never will be, completely standardized. Although immeasurably more valuable in the study of the morphology and morphogenesis of the flagellates, it is nevertheless more difficult to handle than any of the hematoxylin methods of staining.

***Trichomonas prowazeki* Alexeieff, 1909**

(Pls. 13-17)

HISTORY AND TAXONOMY

The only four-flagellate member of the family Trichomonadidae (as defined by Kirby, 1947) known to occur in amphibians was first described by Alexeieff (1909) from two European species of salamanders, *Salamandra maculosa* (= *salamandra*) and *Triton cristatus*, as well as from one anuran species, *Alytes obstetricans*. Alexeieff named the organism *Trichomonas prowazeki*. The same flagellate species was subsequently reported by various writers from a number of other urodeles and anurans.

Most of the authors employed Alexeieff's name *prowazeki* for the species encountered in amphibians, but others used the name *batrachorum* for undoubtedly identical forms. Escomel (1925) reported *Tetratrichomonas batrachorum*, apparently as a new species in the genus *Tetratrichomonas*, from the Peruvian swamp frog, *Telmatobius gebshi* (= *jelskii*?). Bishop (1931) included the four-flagellate trichomonadids from the common European anurans *Rana temporaria* and *Bufo vulgaris* (= *bufo*) in *Trichomonas* (= *Tritrichomonas*) *batrachorum* Perty, 1852 (see figs. 1 and 4 on pl. VI in Bishop's paper). In her paper she stated: "*T. batrachorum* is described as having three anterior flagella of unequal length. . . . In my cultures, however, of *T. batrachorum* from both the frog and the toad flagellates with three or four anterior flagella were present in approximately equal numbers. These flagella were of equal length. . . . Alexeieff (1909) described a *Trichomonas* (*T. prowazeki*) . . . , which differed from *T. batrachorum* in that it had four flagella of unequal length." During the present investigation I encountered mixed infections of *Tritrichomonas batrachorum* and *Trichomonas prowazeki* in intestinal smears of several North American frogs and toads and in cultures of intestinal contents of two bullfrogs. In the commonly employed preparations, stained with iron hematoxylin or iron hematein, often neither the parabasal apparatus nor the anterior flagella are demonstrated with clarity, and this may lead to confusing the three-flagellate *T. batrachorum* with the four-flagellate *T. prowazeki*, but the use of protargol-treated material precludes any such error. Some representatives of the four-flagellate species have subequal anterior flagella; on the other hand, the anterior flagella of *T. batrachorum* are often definitely unequal. In addition to some less conspicuous morphological differences, the two trichomonad species differ greatly in the appearance of their parabasal apparatus, which is discoid in *T. prowazeki* (see below) and V-shaped in *T. batrachorum* (see Honigberg, 1950).

Alexeieff (1910, 1911, 1914a) described and figured trichomonads from the sea bream, *Box salpa*, and from the horse leech, *Hacmopsis sanguisuga*; these trichomonads in all respects apparently resembled *T. prowazeki*. He placed the organisms in the same species with the congeneric symbiotes of amphibians. There are no additional records of *T. prowazeki* from either fishes or leeches. Although it is possible that Alexeieff might have dealt with forms morphologically indistinguish-

able from the trichomonad of amphibians, it seems doubtful that a single species would occur in the various amphibians, in a marine fish, and in a hirudinid annelid. Until more research is done on the intestinal fauna of fishes and leeches we must regard the records of Alexeieff concerning the occurrence of *T. prowazeki* in *Bor salpa* and *Haemopsis sanguisuga* with a great deal of caution.

Parisi (1910) reported *Trichomonas prowazeki* from the marsh crocodile, *Crocodylus* (*Crocodylus*) *palustris*. At the same time he created a new subgenus *Tetratrichomonas* in the genus *Trichomonas*, to include the trichomonads equipped with an undulating membrane and four anterior flagella. Alexeieff (1911) raised *Tetratrichomonas* to generic status. However, in his 1912 paper he referred to the four-flagellate *Trichomonas brumpti* from the Ceylon terrapin thus: "*Trichomonas* appartient au sous-genre *Tetratrichomonas* Parisi."

In his subsequent publications Alexeieff (1914a, b) maintained the subgeneric status of *Tetratrichomonas* Parisi. However, his 1914a paper contains both versions. In two places the flagellate is referred to as *Trichomonas* (*Tetratrichomonas*) *prowazeki*, but on two other occasions the name *Tetratrichomonas prowazeki* is used for it. Kofoid and Swezy (1915), following Alexeieff (1911), placed the species *prowazeki* from the California newt, *Diemictylus* (= *Triturus*) *torosus*, in the genus *Tetratrichomonas*. Goodey and Wellings (1917) placed the four-flagellate trichomonadid, *Trichomonas buccalis* (= *tenax*), occurring in the human mouth, also in the genus *Tetratrichomonas*. Kofoid (1920) pointed out that, since the type species of the genus *Trichomonas*, *T. vaginalis*, reported by Donné (1836) from man, was subsequently found to possess four anterior flagella, the generic name *Trichomonas* should, on the basis of priority, apply to four-flagellate trichomonads with an undulating membrane and that Parisi's (1910) name *Tetratrichomonas* should thus be placed in synonymy with *Trichomonas* Donné, 1836.

Besides the report of Parisi (1910) on *Trichomonas prowazeki* from the crocodile, there is in the literature one more record dealing with a closely comparable flagellate of reptiles. Das Gupta (1935) described *Trichomonas* sp. from a North American colubrid, *Natrix erythrogaster*. As far as can be ascertained from his description and figures, the Indian author probably dealt with *T. prowazeki*.

Das Gupta (1927) recorded a trichomonad with three anterior flagella and a well-developed undulating membrane, from an Indian colubrid, *Ablabes calamaria*. According to his description, a fourth free flagellum passed backward and was closely adjacent to, but not connected with, the undulating membrane. However, the photomicrograph of the flagellate (see pl. 47 in Das Gupta, 1927) suggests that he might actually have seen a torn-off posterior flagellum and mistaken it for a free flagellum. Thus, although Das Gupta (1927, 1936) considered *Trichomonas* sp. from *Ablabes calamaria* to have four free flagella, it is more likely that he actually dealt with a three-flagellate species.

DESCRIPTIVE ACCOUNT

The shape of the body may vary considerably in a single population. It apparently depends upon the age of the organisms (the youngest, small individuals usually being stouter), upon the amount of solid food present in the cytoplasm, and probably upon other, as yet unknown, factors. However, the examination of both living

and fixed organisms seems to indicate that the elongate type is more frequent (pl. 13, *a*, *c*). The giant individuals often tend to be almost spherical.

The cytosome appears to be quite elastic. When an organism passes between two closely adjacent piles of solid material such as masses of rice-starch grains or accumulations of bacteria and debris, the width of the body may in some parts be reduced almost to that of the axostyle. However, as soon as the flagellate reaches a free space the shape of the body is restored.

For purposes of comparison several sets of measurements of various amphibian and reptilian strains are given. In each set the results are based on measurements of fifty nondividing individuals of a single population. Body length $10.5(6.5-16.5)\mu$ in *Triturus torosus*; $10.5(6.5-15.5)\mu$ in *Rana boylii*; $10(7-22)\mu$ in *Rana catesbeiana*; $8.5(5.5-13)\mu$ in *Gehyra* sp.; $12(6.5-21)\mu$ in *Thamnophis elegans atratus*; and $10.5(6.5-22)\mu$ in *Thamnophis ordinatus infernalis*. Body width $5.5(4.5-7.5)\mu$ in *Triturus torosus*; $6.5(4.5-10)\mu$ in *Rana boylii*; $6(4-18.5)\mu$ in *Rana catesbeiana*; $5.5(3.5-11)\mu$ in *Gehyra* sp.; $7.5(4.5-15.5)\mu$ in *Thamnophis elegans atratus*; and $6.5(4.5-18.5)\mu$ in *Thamnophis ordinatus infernalis*.

All these measurements are closely comparable. The same is true of the measurements made from other populations of the strains listed above, as well as from different strains from various amphibian and reptilian hosts belonging to the aforementioned species or to different species (*Bufo terrestris* and *Gekko vittatus*). In all the strains nondividing organisms exceeding 15μ in length are rare and may be regarded as giants.

The measurements of living organisms seem to indicate that there is a considerable amount of shrinkage due to fixation.

Parisi (1910) recorded the size of *Trichomonas prowazeki* from *Crocodylus* (= *Crocodylus*) *palustris* as $15-25\mu \times 7-15\mu$. The measurements of the organisms represented in his figures (pl. 14, figs. 1-5, in Parisi, 1910) are comparable to the measurements given in his text. Alexeieff (1911) reported this trichomonad species from *Salamandra maculosa* (= *salamandra*), *Triton cristatus*, and *Alytes obstetricans* to be $10-14\mu \times 4-7\mu$. Kofoid and Swezy (1915) gave the range of the body length in the strain from *Triturus torosus* as $12-25\mu$, with most individuals not exceeding 20μ . However, a typical organism represented in figure 68 on plate 6 in their paper (Kofoid and Swezy, 1915) measures $10.5\mu \times 7.5\mu$. The measurements reported by Escomel (1925) for *Tetratrichomonas batrachorum* (= *T. prowazeki*) from *Telmatobius gebbski* (= *jelskii*?) are $12-15\mu \times 7-8\mu$. Finally, Das Gupta (1935) stated that the four-flagellate *Trichomonas* from the colubrine snake *Natrix erythrogaster* ranged in length from 14μ to 20μ . On the other hand, the organisms represented in figures 1-7 on plate 2 in his paper measure $11.5-13.5\mu \times 7-8.5\mu$.

In general, the measurements given by the afore-mentioned authors correspond to those obtained in the present investigation. The range of the body length as given by them always falls to a greater or less extent in the upper part of the range noted in the strains I studied. If we assume that the magnifications of the figures in the papers of Kofoid and Swezy (1915) and Das Gupta (1935) are accurate, the length of the presumably typical flagellates represented in these figures corresponds more closely to that reported in the present paper than to the length which these authors give in text. The omission of the smaller organisms from the size

range could sometimes (e.g., Escomel, 1925) have been due, perhaps, to inclusion of the axostylar projection in the measurements. Also, occasionally, the method of fixation could have been responsible, in part at least, for the size differences. Furthermore, it is certain that natural size variations also exist among different strains. However, even the most extreme quantitative differences among the strains of *T. prowazeki*—as exemplified by those between the strain from *Crocodylus palustris*, which according to Parisi (1910) measured $15.25\mu \times 7.15\mu$, and that from *Gehyra* sp., the smallest population of which ranged from 5.5μ to 10μ in length and from 3.5μ to 7.5μ in width—do not approach the quantitative differences existing among the various strains of *Trichomonas termopsidis*. Andrews (1925) thought that the different size groups, 11–55 μ long and 50–150 μ long, should be assigned to distinct races or perhaps even distinct species. However, later investigations (see Kirby, 1931), based on a statistical analysis of a large sample of organisms, proved that no “size races” existed among twelve hundred individuals of *T. termopsidis*, the length of which ranged from 9μ to 85μ . Similarly, the size range ($7\text{--}22\mu \times 4\text{--}18.5\mu$) of a single population of *T. prowazeki* from *Rana catesbeiana* nearly covers the combined size range of the largest strain from the crocodile and the smallest strain from the gecko.

There are almost invariably four anterior flagella, which originate in the anterior part of the blepharoplast complex (pls. 13, *d*; 14, *c–f*). The flagella are united in a short whip or column at their base (pls. 13, *a* and *b*; 14, *a, b, d–g*) and terminate in knobs or rods (pl. 13, *b* and *c*). At times the rods may be situated at an angle to the long axis of the flagella (pl. 13, *c*). Flagellar counts were made in the strains from *Rana catesbeiana*, *Thamnophis elegans atratus*, and *Thamnophis ordinatus infernalis*. One population of each of the three strains was employed. In the first population, out of 200 solitary, nondividing individuals in which the flagella could be clearly seen, 98.5 per cent showed four, and 1.5 per cent only three flagella. In the second, of 400 similar individuals, 94 per cent had four, and 6 per cent only three flagella. Finally, in the third population, of 200 individuals, 98 per cent exhibited four, and 2 per cent only three flagella. Usually, the three-flagellate organisms are quite small. In most individuals the four flagella are unequal to subequal in length (pls. 13, *a, c*; 14, *a* and *b*). In some organisms, as mentioned by Alexeieff (1909), the flagella appear to consist of two groups. Each group contains two flagella. One group is shorter than the other and within each the flagella are subequal (pls. 13, *b*; 14, *b*). The inequality of the anterior flagella may be explained by their behavior in division (see the discussion of morphogenesis below). The longest (full-grown) flagella measured in twenty-five individuals of a single population of the strains from *Rana catesbeiana* and *Thamnophis elegans atratus* ranged in length from 18.5μ to 27.5μ and from 18.5μ to 24μ respectively, averaging 22.5μ and 20.5μ .

The movement of the anterior flagella could be easily analyzed in the living organisms, which, slowed down by being kept forty-eight to seventy-two hours at room temperature on paraffin-sealed slides, were examined in dark field. The pattern of the movement does not differ from the one described by Kirby (1943) in *Pentatrichomonas hominis* and by Honigberg (1947) in *Monocercomonas verrens*. The flagella, after a momentary rest on the ventral surface of the cytosome, are

thrown simultaneously forward and dorsad. After the rapid forward sweep they return gradually one after another to their ventral position. During their travel back the flagella become curved, their concavity facing the ventral side of the cytosome.

The costa and the undulating membrane originate in the dorsal part of the blepharoplast complex (pls. 13, *d*; 14, *c* and *d*). Both of them reach the posterior end of the cytosome and either do not spiral or exhibit a very slight counterclockwise twist (pls. 13, *a-c*; 14, *a*). The costa is a slender rod, slightly wider than the anterior flagella (pls. 13, *a-c*; 14, *a* and *b*). It tends to be somewhat attenuated at the posterior end (pls. 13, *b*; 14, *b*).

The undulating membrane exhibits several conspicuous waves (pls. 13, *a, c*; 14, *a*). Its free margin appears ribbon-shaped in many of the more heavily impregnated specimens (pls. 13, *d*; 14, *b-d*). In suitable impregnations the margin may be seen to consist of an inner filament and the outer, attached part of the posterior flagellum (pls. 13, *b* and *c*; 14, *a*). The double nature of the free margin may also be observed in living organisms examined in dark-field illumination. The posterior flagellum of the acroneme type continues for a considerable distance beyond the end of the undulating membrane (pls. 13, *b* and *c*; 11, *a*). In the strains from *Rana catesbeiana* and *Thamnophis elegans atratus* the free part of the posterior flagellum ranges in length from 7.5μ to 13μ , averaging 10μ .

The parabasal apparatus, situated dorsally and to the right of the nucleus (pls. 13, *a-c*; 14, *a, e, f*), originates in the blepharoplast complex somewhat to the right of the undulating membrane and the costa (pls. 13, *d*; 14, *c*). It consists of a discoid parabasal body and two distinct filaments (pl. 14, *a*). The disk may show various deformations (pl. 13, *b, e, f*), but it is typically circular (pls. 13, *a, c*; 11, *a*). The diameter of the disk averaged about $1.75(1.5-2)\mu$ in the strain from *Rana catesbeiana* and about $2(1.5-2.5)\mu$ in the strain from *Thamnophis elegans atratus*. In well-impregnated preparations the disk shows a dark, conspicuous margin and an equally dark central granule (pls. 13, *c*; 11, *a*). Undoubtedly the great affinity to protargol exhibited by the central granule and the margin of the disk reflect their structural differentiation. The space between the margin and the granule may take various amounts of impregnation. Thus it may be very light (pls. 13, *c*; 11, *a*), rather dark (pl. 13, *b*), or at times as dark as the margin and central granule (pl. 16, *e*). These variations are due primarily to largely uncontrollable factors of technique, which may affect differently various areas of a single smear; however, intrinsic differences in structure may also be involved. In dorsal and ventral views, the parabasal body appears as a more or less stout rod (pl. 14, *h*), which often seems to have a double edge (pls. 13, *d*; 14, *e* and *f*).

The parabasal body is bordered anterodorsally by a conspicuous filament, the "major" parabasal filament (pls. 13, *g*; 14, *f, h*), which connects the disk to the blepharoplast complex (pl. 14, *f*). The distance between the parabasal body and the blepharoplast is very small (pl. 14, *e* and *f*), and often the disk may appear to originate directly in the latter structure without the intervention of the parabasal filament (pls. 13, *b*; 14, *a*). The "major" filament continues posterior to the parabasal body for about $6.5-10\mu$, reaching at times almost the posterior end of the cytosome (pls. 13, *b* and *c*; 14, *a* and *b*). Besides the "major" filament there is an-

other, more slender and shorter filament associated with the disk. This "minor" parabasal filament is situated near the ventral side of the parabasal body (pls. 13, *b, f, g*; 14, *a, b, c-j*). It may be seen in many, but not in all, of the protargol-treated organisms. The length of the "minor" filament is about $\frac{1}{3}$ – $\frac{1}{2}$ that of the "major" filament.

Some of the larger flagellates of both the amphibian and reptilian strains show greatly enlarged parabasal bodies (2 – $3.5\mu \times 1.5$ – 2μ), which often exhibit two central granules instead of one (pls. 13, *g*; 14, *b, g, i, j*). At times these enlarged parabasals appear to be more or less definitely constricted midway between the two granules, at right angles to the longer axis of the disk (pls. 14, *b*; 17, *d*). The flagellates which possess such enlarged disks fail to show any indication of division; on the other hand, one of the two parabasals in organisms which are in early division stages may exhibit two central granules (pl. 15, *g*). On a few occasions a parabasal disk belonging to one of the two fully developed mastigonts of a double organism showed two central granules and the median constriction (pl. 17, *d*).

The presence of the enlarged parabasal disks with two central granules and occasional constrictions in a small number of individuals of *T. prowazeki* resembles the situation in *T. termopsidis*, in which species Kirby (1944) reported branched rather than typically single parabasal bodies in some organisms. Kirby and Honigberg (1950) found at times two granules instead of the usual single granule of parabasal material applied to the parabasal filament in *Pentatrichomonas hominis*.

Although the parabasal apparatus cannot be demonstrated in most preparations stained with iron hematein, it shows clearly in some organisms treated in this way (pl. 13, *a*). In all, only the disk can be seen, and even then, neither the dark rim nor the central granule is visible.

The axostyle is differentiated into the capitulum and the trunk. The capitulum is a spatulate expansion of the anterior third of the axostyle and appears to be connected to the blepharoplast complex (pl. 14, *c* and *d*). It is applied to the ventral part of the left surface of the nucleus (pl. 14, *c-f*) and narrows gradually into the trunk some distance posterior to the nucleus (pls. 13, *a-c*; 14, *a, c, d*). The width of the capitulum is about three times the diameter of the axostylar trunk. The ventral margin of the capitulum may at times impregnate quite heavily with protargol or take a deep hematoxylin or hematein stain. Under those circumstances the relatively clear part of the capitulum between its heavy ventral margin and the nucleus could be mistaken as representing the "cytostome" (pl. 13, *a, c*).

The trunk of the axostyle is a hyaline rod about 0.3 – 0.4μ in diameter (pls. 13, *a-c*; 14, *a*). After traversing the cytosome the axostyle projects from the posterior end for 3.5 (2 – 5) μ in the strain found in *Rana catesbeiana* and for 4 (2 – 7.5) μ in the strain obtained from *Thamnophis elegans atratus*. The axostyle shows neither axostylar granules nor periaxostylar rings. In most fixed preparations its projecting part appears to taper gradually to a point (pls. 13, *a-c*; 14, *b*), which at times may be prolonged in a fine, short filament (pl. 13, *a*). In protargol-treated preparations this part takes a rather heavy impregnation.

Certain details concerning the axostyle may best be ascertained through a study of living organisms in dark field. The projection of the axostyle appears to be quite stiff and constant in length in a given organism. The length does not change with

the contractions of the cytosome; thus it is likely that these contractions are possible through bending of the intracytoplasmic part of the axostylar trunk at some point. The behavior of the relatively slender axostyle during contractions could not be traced in *T. prowazeki*; however, it is probably not unlike that described by Kirby (1931) for the axostyle of *Trichomonas termopsidis*. Although in fixed and stained preparations the projection of the axostyle appears to taper rather gradually, observations of living organisms suggest that this gradual tapering of the axostylar projection may not occur in life. A dark-field study indicates that the projecting part does not taper until almost its very end, and then it comes abruptly to a point, the terminal segment therefore being wedge-shaped in an optical section. In some organisms the axostyle may be continued in a fine filament.

The pelta is a transverse, crescent-shaped membrane situated anterior to the nucleus and close to the surface of the cytosome (pls. 13, *a-d*; 14, *a-g*). Its ventral part originates near the anterior segment of the axostylar capitulum and appears to be connected to it (pls. 13, *d*; 14, *c* and *d*). It seems quite probable that the pelta is a direct extension of the axostyle. If it is not a direct extension, at least it remains in a very close spatial relationship to the capitulum. The membrane runs dorsad and to the right at an angle to the anteroposterior axis of the cytosome (pls. 13, *d*; 14, *c-f*). Its dorsal part tapers gradually to a point (pls. 13, *b-d*; 14, *c, d, f*) and finally ends in a slender filament, which may vary in length (pls. 13, *b, d*; 14, *c, e, f*). The tapering part tends to turn to the left (pl. 14, *c, e, f*). When seen from the right side, the pelta often resembles a cap, which in protargol-treated specimens takes a dark impregnation (pl. 13, *b* and *c*). In many organisms the membrane appears to be rather slender (pls. 13, *b*; 14, *c, f*); in others it is a little stouter (pl. 13, *c* and *d*). Some of the large organisms which exhibit the enlarged parabasals with two central granules seem also to possess a wide and often somewhat disfigured pelta (pl. 14, *b, g*). Although the pelta may be best demonstrated in protargol-impregnated preparations, it also shows in some of the flagellates stained with iron hematein (pl. 13, *a*).

The nucleus is ellipsoidal or ovoidal (pls. 13, *a* and *b*; 14, *a, c, e, f*), and averages $3(2-3.5)\mu \times 2(1.5-2.5)\mu$ in the strain from *Rana catesbeiana* (in one giant organism, $5.5\mu \times 3.5\mu$) and $3.25(2-4)\mu \times 2.25(1.5-3.5)\mu$ in the specimens obtained from *Thamnophis elegans atratus*. The long axis of the nucleus coincides with the long axis of the cytosome (pls. 13, *a*; 14, *a*) or may be at a slight angle to it (pl. 13, *c*). The interphase nucleus (pl. 13, *a*) is filled with finely distributed chromatic granules, but, except for the usually single, spherical endosome, shows no large accumulation of dark-staining material. The endosome is always surrounded by a clear halo. At times two endosomes may be found in a nucleus.

Many organisms stained with iron hematoxylin or hematein show numerous dark spherical inclusions of different sizes (pl. 13, *a*). The smaller inclusions represent, in part at least, ingested bacteria. On the other hand, all the larger and some of the smaller spheres are probably metabolic products. The rice-starch granules, which may fill the cytoplasm of many culture-grown flagellates, do not take any stain.

One series of preparations of the cultivated strain from *Thamnophis elegans atratus* contained a number of large organisms which at times showed smaller, as yet little-digested, flagellates of the same species in their cytoplasm (pl. 14, *b*).

Since no similar specimens were ever observed in the permanent preparations of the remaining strains and no active cannibalism was encountered in the numerous dark-field examinations of living organisms from the culture-grown strains, it appears that cannibalism is rather rare.

Dark-field observations brought out certain hitherto unobserved details concerning the method of feeding among trichomonad flagellates. The phenomenon described below was observed in all the cultivated strains of *T. prowazeki* and in several strains of *Tritrichomonas batrachorum* from amphibian and reptilian hosts.

On many occasions when a flagellate brushes past an accumulation of rice-starch grains or bacteria, a fine filamentous projection of the cytoplasm is extended from the posterior region of the cytosome in close proximity to the point at which the axostyle emerges from the body. A grain of rice starch or one to several bacteria are caught by the end of the cytoplasmic projection, which is then retracted. The food particle is taken in at the posterior end of the cytosome and remains for some time in the vicinity of the point at which it was incorporated into the cytoplasm. The whole process of extension and retraction of the cytoplasmic filament is very rapid. It appears as though a fine needle were pushed out and immediately drawn back. Since this process was observed on numerous occasions in various populations of different strains, it undoubtedly constitutes a commonly employed method of feeding in the two trichomonad species in which it was seen. Further search will probably uncover a similar feeding method in other trichomonads which are capable of ingesting solid food particles.

In spite of a long series of observations I have never noted any food intake at the anterior end of the cytosome in either *Trichomonas prowazeki* or *Tritrichomonas batrachorum*.

MORPHOGENESIS

DARK-FIELD OBSERVATIONS

On several occasions I was successful in following the latter part of the division process in living organisms of the strain from *Thamnophis elegans atratus* by means of dark-field illumination.

In two specimens division could be followed from the stage at which there were still a single elongate nucleus, two anterior flagella at each of the poles, and two almost equally well-developed undulating membranes. Although no nuclear details could be seen, the flagellates must have been in anaphase. On both occasions the division was timed. Since only a two-minute difference was noted in the time intervening between the moment at which these two organisms were first sighted and that at which the daughter nuclei were formed, it is assumed that the division process was approximately equally advanced in the two flagellates at the start of the observation. The four flagella (two at each pole) and the two undulating membranes were in constant motion, and although the dividing organisms did not progress for an appreciable distance in any direction, they were continually rotating. Not much change could be observed for ten minutes on one occasion and for twelve minutes on the other. Then rather suddenly the nucleus started to elongate rapidly. In less than one minute it went through the stages figured in plate 16, *f* and *g*, two nuclei being ultimately formed. At that time a short and practically immobile stump of the third anterior flagellum (see pl. 17, *a* and *b*) could be ob-

served at each pole. Because of the very fast movement of the long parental flagella and the constant rotation of the cytosome, the development and activity of the third flagellum was difficult to follow. It was impossible to be sure whether in either organism the stumps appeared simultaneously at both poles, but the observations of fixed and stained material suggested that in most individuals this was true. The difficulties notwithstanding, the study of the two afore-mentioned dividing organisms and of three additional specimens in more advanced stages of division brought out certain facts concerning the behavior of the growing flagella. Although the very early stumps were practically immobile, the somewhat longer young flagella exhibited the usual movements. These movements were slower than those of the long parental flagella. Not until the young flagella reached a considerable length did their activity become synchronized with that of the parental counterparts.

For some time the organism, although pulled in one or another direction by each of the two mastigonts, remained rather broadly oval. As the division progressed the mastigonts moved farther and farther apart. The action of the mastigonts and the cytoplasmic contractions caused a continual change in the shape of the cytosome, which was more elongate at some times than at others.

About fifteen to seventeen¹ minutes after the formation of the daughter nuclei the two mastigonts were at a 180° angle to each other. The tendency for elongation progressively increased. In about seven to nine minutes the organism assumed the shape of a frankfurter sausage, being similar to the one represented in figure *g* on plate 20, but somewhat more elongate.

After about three to four minutes the body contracted suddenly. Almost simultaneously a shallow furrow appeared midway and at right angles to the poles. The furrow continued to deepen more or less uniformly around the equator of the cytosome. During the deepening of the furrow the cytoplasmic activity was quite pronounced and the mastigonts remained in constant rapid motion, pulling in opposite directions. The segment of the cytoplasm connecting the two daughter individuals decreased rapidly in diameter. In less than three minutes there was only a fine cytoplasmic strand connecting the two organisms, which pulled farther and farther apart, swimming in one direction and then in another. In about 3.5 to 4.5 minutes after the onset of cytosomal division the connecting strand was broken, and small, rather stumpy young flagellates swam rapidly apart.

Invariably only one of the young individuals could be followed. Each of the organisms observed possessed three active flagella, one of which was shorter than the others. The rapid movements of these flagella did not permit observation of the short fourth flagellum, which on the basis of the study of fixed and stained preparations was undoubtedly present in this stage. Further attempts will be made in the future to follow the development of the daughter flagella *in vivo*.

It is apparent from the above description that the cytosomal division of *T. prowazeki* does not follow the pattern, exhibited by various flagellates, in which a longitudinal furrow starts at the anterior end and proceeds toward the posterior

¹ The numbers of minutes in this and other places in the following description indicate the periods noted for each of the two dividing organisms. Although in these particular observations the periods were closely comparable, it is probable that if more dividing forms were examined the variation in time at which certain morphogenetic changes take place would be found to be much greater.

end of the cytosome. As pointed out by Kirby (1944), cytosomal fission in many trichomonads is a modification of the longitudinal type.

The study of division in living organisms explains the relative proportions of the various division stages as seen in fixed preparations. Since the animal appears to remain for a relatively long time in the stage in which the daughter nuclei are already present, this stage is quite frequent in the permanent slides. On the other hand, the rather short period during which cytoplasmic fission takes place is closely correlated with the infrequent appearance of organisms in cytokinesis in the fixed preparations. No such stages were seen during the present study, and only a few figures of flagellates in cytokinesis may be found in the available literature dealing with the various genera and species of trichomonads. Figure 37 on plate 4 in Samuels' (1941) paper on *Tritrichomonas augusta* probably represents an organism at the onset of cytosomal division. Kirby (1944, figs. 23 and 24 on pl. 3) illustrates *Devescovina lemniscata* in an earlier and a more advanced stage of cytokinesis respectively. Whereas no early stages of division could be followed in vivo, their relative frequency of occurrence must also be correlated with the time span during which they take place. Thus the reorganization of the parabasal apparatus, which is very difficult to follow in the permanent slides, is undoubtedly accomplished very rapidly.

STUDY OF PROTARGOL-TREATED PREPARATIONS

Although the study of morphogenesis in organisms treated with strong protein silver has many advantages, its greatest drawback lies in the fact that the morphogenetic changes affecting the various mastigont organelles cannot be correlated with the simultaneous occurrences in the nucleus. Also, the behavior of the blepharoplast, particularly in the early stages of division, cannot be followed. Although the hematoxylin-stained preparations are admittedly more suitable for demonstration of the blepharoplasts, as pointed out by Kirby (1944), "not much can be seen of what actually has happened [to the blepharoplast group in early division] in the small trichomonads that have been the ones most studied."

Since the intranuclear changes cannot be seen in protargol preparations, in the following description I shall avoid designating the stages at which certain morphogenetic phenomena take place by the commonly employed terms of pro-, meta-, ana-, and telophase.

Anterior flagella.—The four parental flagella are equally distributed between the daughter blepharoplasts (pls. 15, *h*; 16, *a-g*). At times one of these flagella may be much shorter than the remaining three (pl. 16, *a* and *b*), indicating that division probably set in before the youngest flagellum could reach any great length.

Most of the descriptions of division in various species of the genus *Trichomonas* (Martin and Robertson, 1911; Kofoid and Swezy, 1915; Andrews, 1925; Hinshaw, 1926; Bishop, 1931; Kirby, 1931, 1944; Powell, 1936; Kirby and Honigberg, 1950) are in agreement with the foregoing observation.

The short stump of the third flagellum appears much later. It was never seen in division stages earlier than those in which the two daughter nuclei were already formed. However, the stumps seem to be present at both poles in all the organisms at the afore-mentioned stage of division (pl. 17, *a* and *b*).

The fourth flagellum appears still later, but since it may be found in some dividing organisms prior to cytokinesis (pl. 17, c) and since the percentage of three-flagellate, solitary (nondividing) forms is extremely low in all populations, it seems that this flagellum is usually present before the cytosomal division takes place. The population from *Thamnophis elegans atratus* which contained the largest percentage (6 per cent) of three-flagellate organisms showed also the largest number of division stages. Thus it is likely that, when the division rate is high, the cytosomal division may occasionally take place prior to the time at which the full complement of anterior flagella is restored.

In many small, presumably young organisms one of the anterior flagella appears to be quite short in comparison with the remaining three. The same situation can be found occasionally in the larger individuals, which may enter division even before the fourth flagellum achieves much length. On the other hand, in a great many of the average-sized organisms the fourth flagellum is almost as long as the third. Thus it appears that more often division does not take place until the fourth flagellum almost equals the third.

The morphogenetic process whereby the full complement of anterior flagella is restored in *T. prowazeki* appears to be closely comparable to that in *Trichomonas vaginalis* as described by Powell (1936). It also resembles the situation reported by Hinshaw (1926) in *Trichomonas tenax*, by Kirby and Honigberg (1950) in *Trichomonas guttula*, and observed by me in *T. brumpti* (see below). However, although in the former two species the full complement of anterior flagella is present prior to cytokinesis, in *T. guttula*, in *T. brumpti*, and, according to Hinshaw (1926), also in *T. tenax*, the fourth flagellum appears after the cytosomal division has taken place.

The five afore-mentioned species differ from *Trichomonas termopsidis*, in which, according to Andrews (1925) and Kirby (1931, 1944), the two new flagella appear at each pole while the nucleus is still in the prophase stage. Consequently, due to the early outgrowth of the new flagella, all the solitary organisms exhibit four long flagella.

In many devescovinids, in practically all members of the genus *Tritrichomonas* in which division has been carefully studied, and in some members of the genus *Trichomonas*, the full complement of anterior flagella is present prior to cytokinesis; but in other species of the latter genus such is not the case. Possibly the species which exhibit the delayed regeneration of the fourth flagellum reflect the phylogenetic history of the genus *Trichomonas* Donné.

Parabasal apparatus.—The reorganization of the parabasal structures in division is, beyond any doubt, the most difficult process to follow in trichomonads in which the parabasal apparatus is small. Furthermore, judging from the relatively very small number of dividing organisms which throw any light on the early morphogenesis of the parabasal, this process must proceed quite rapidly. The following account is far from being complete; nevertheless in all essential points it agrees with the description of reproduction of the parabasal apparatus in larger trichomonads, in which this organelle is far more conspicuous.

The first signs of reorganization of the parabasal apparatus of *T. prowazeki* appear very early in division, when the new costa is forming. According to Kirby

(1944), the process does not start in *Trichomonas termopsidis* and in many devescovinids before two daughter blepharoplasts are present. On the other hand, Grassé and Faure (1935, 1939) found that in *Tritrichomonas caviae* a new parabasal appears prior to the division of the blepharoplast. Since the early development of the blepharoplast complex could not be observed in the protargol-impregnated material, it is impossible to tell which of the above situations prevails in *Trichomonas prowazeki*.

The earliest indications of reorganization of the parabasal apparatus in the presently studied material were encountered in division stages which showed the somewhat distorted, invariably very darkly impregnated, and thus perhaps partly dedifferentiated parental parabasal disk at some distance posterior to the blepharoplast (pl. 15, *a-c*). Sometimes there is no trace of parabasal material in the vicinity of the blepharoplast (pl. 15, *a* and *b*); at other times a certain amount of parabasal material may be seen at the blepharoplast (pl. 15, *c*). It is possible that the whole, relatively small, old parabasal body is always discarded and that the material seen at the blepharoplast is neoformed. On the other hand, it is equally possible that, occasionally, at least, in conformity with all the other trichomonads in which morphogenesis of the parabasal apparatus has been studied (see Kirby, 1933, 1936, 1938, 1944; Grassé and Faure, 1935, 1939; Grassé, 1937), part of the old parabasal material remains attached to the blepharoplast. If this were true, the situation in *T. prowazeki* would be similar to that reported in *T. termopsidis* by Kirby (1944).

Between the blepharoplast region and the discarded parabasal there are usually two slender fibrils (pl. 15, *b* and *c*), but at times a single, stout, darkly impregnating one (pl. 15, *a*). These fibrils represent the new "major" parabasal filament and the new costa. When a single stout fibril is seen, it undoubtedly consists of the aforementioned two organelles, which are merely closely applied to each other. This spatial relationship is maintained in some of the later division stages (pl. 15, *d*). Although it is impossible to be sure whether the new "major" parabasal filament is entirely or only partly neoformed, the fact that one of the new small parabasal disks exhibits a stouter and longer "major" filament in some of the subsequent division stages (pls. 15, *d, e, g, h*; 16, *a*) favors the view that this filament is only partly neoformed.

The development of the "minor" parabasal filament cannot be followed with any degree of certainty. The filament does not appear to be present in either of the very young parabasal apparatuses (pl. 15, *d* and *e*), but it may be seen in somewhat more advanced stages of their development (pl. 15, *g* and *h*). It is thus possible that this structure may be entirely neoformed in both daughter parabasal complements.

The discard, including a segment of the "major" parabasal filament, perhaps the whole "minor" parabasal filament, and at times probably only a major part but at other times the whole parabasal disk, appears to disintegrate quite rapidly. Only in a single instance did I see a trace of the discarded parabasal disk in a division stage in which the two small neoformed parabasals were already present (the stage corresponded to the one represented in fig. *d* or *e* on pl. 15). The discard was far removed from the blepharoplast and very small.

The two daughter parabasal disks are well differentiated before the blepharoplasts move noticeably apart (pl. 15, *d, e, g*). For the remainder of the division

process the parabasals merely increase in size and develop a full complement of fibrils (pls. 15, *h*; 16, *a-g*; 17, *a-c*).

Costa.—As in all other members of the family Trichomonadidae, the parental costa remains intact and becomes incorporated into the mastigont system of one of the daughter individuals. The other individual receives a neoformed costa. A review of earlier works on this subject may be found in Bishop's (1931) paper, and some of the later reports are mentioned by Kirby (1944).

The outgrowth of the new costa constitutes one of the earliest indications of division in protargol preparations in which the nuclear contents cannot be studied. Whether the origin of this organelle precedes the division of the blepharoplast could not be ascertained in the available material. Since, however, according to the recent investigations by Grassé and Faure (1935, 1939), Samuels (1941), and Kirby (1944), the organelle does originate before the division of the blepharoplast in *Tritrichomonas caviae*, *Tritrichomonas augusta*, and *Trichomonas termopsidis*, it is likely that in *Trichomonas prowazeki* also the costa grows out prior to division of the blepharoplast complex.

Most of the earliest division stages in which the new costa was seen also showed the first steps of the reorganization of the parabasal apparatus (pl. 15, *a-c*). The early development of the neoformed costa is at times difficult to follow, because the organelle remains in a close spatial relationship to the growing "major" parabasal filament. Whereas usually the two filamentous structures are more or less separate (pl. 15, *b-e*), on some occasions they are closely applied to each other throughout their whole length (pl. 15, *a*). In the latter condition only the relative stoutness of the apparently single filament indicates its double nature. When the two filaments are more completely separate the longer and more peripheral one represents the costa (pl. 15, *c, e*). In somewhat later stages of division the new costa may appear considerably stouter than the "major" parabasal filament associated with the more dorsally located parabasal apparatus (pl. 15, *f*).

As division progresses the new costa increases in both length and width (pl. 16, *a-d*). Although the exact time in the division process at which the neoformed costa may reach its full development varies from individual to individual, the new costa often almost equals the parental homologous organelle in both length and width prior to the stage at which the nucleus shows any sign of constriction (pl. 16, *e*).

Undulating membrane.—The parental undulating membrane remains intact during division and with the parental costa is associated with one of the two daughter blepharoplasts. The other undulating membrane is neoformed independently of the new costa.

These findings are in essential agreement with the reports of many authors (among others, Wenyon, 1907; Kuczynski, 1914, 1918; Cutler, 1919; Tanabe, 1926; Bishop, 1931; Powell, 1936; Samuels, 1941; Kirby, 1944) on the behavior of the parental and new undulating membranes in division in various members of the family Trichomonadidae.

The development of the new undulating membrane constitutes the least understood morphogenetic process in trichomonads. Most authors agree that the membrane develops in connection with the posterior flagellum, but they leave the mechanics of the process largely unexplained. The present investigation revealed

certain hitherto unknown steps involved in the formation of the new undulating membrane in *T. prowazeki*.

The earliest division stage in which the new posterior flagellum could be observed also exhibited a short new costa and two well-formed parabasal disks (pl. 15, *d*). This flagellum originates definitely apart from the four anterior flagella and terminates in an acroneme. No five-flagellate nondividing organisms were seen in any population of the various strains of *T. prowazeki*. Furthermore, the fifth flagellum encountered in early division stages ends in an acroneme, which is characteristic of the posterior flagellum of this and other trichomonad species (see Kirby, 1945; Honigberg, 1947; Kirby and Honigberg, 1949*a*, 1950), rather than in a knob or rod, which are the customary endings of the anterior flagella in trichomonads (see the works just mentioned, and also Wenrich and Saxe, 1950). Thus, there is no reason to believe that the fifth flagellum merely represents a supernumerary anterior flagellum.

In all the organisms which were in early division stages and already showed the posterior flagellum, this flagellum was found to be relatively long (pl. 15, *d*). Thus it seems reasonable to assume that this organelle actually originates at still earlier stages of division than those in which it was seen, probably very soon after the first appearance of the anlage of the costa. If this were true, then the time of origin of the posterior flagellum in *T. prowazeki* would correspond closely to that specified by Samuels (1941) and Kirby (1944) for the new undulating membrane in *Tritrichomonas augusta* and *Trichomonas termopsidis* respectively. In both of the latter species the primordium of the undulating membrane appears in the prophase, soon after the division of the parental blepharoplast is completed.

As the division of *T. prowazeki* progresses the posterior flagellum appears to grow very rapidly (pl. 16, *a*) and soon achieves a relatively great length (pl. 16, *b*). In the division figures encountered in the available material the earliest indication of the undulating membrane is a widening of the proximal part of the long posterior flagellum (pl. 16, *a* and *b*). The ribbon-shaped proximal part of the flagellum is twice as wide as its distal, far longer, segment. At times a sheath of cytoplasm (the undulating membrane proper) may be seen along the inner border of the ribbon (pl. 16, *d*). In time, progressively larger segments of the flagellum become ribbon-shaped (pl. 16, *c-g*). The widened part sometimes appears to consist of two filaments (pl. 16, *d*); the outer filament continues in the free posterior flagellum, whereas the inner one represents the differentiated margin of the undulating membrane. On some occasions the posterior flagellum may be torn off the distal part of the marginal filament (pl. 16, *e*). The partial separation of the two marginal elements could be seen at times also in the parental undulating membranes (pl. 16, *d*).

In the earlier stages of division the developing undulating membrane appears not to be attached to the new costa (pl. 16, *a, c*). However, it soon becomes thus attached (pl. 16, *d?*, *e-g*). In this respect the development of the membrane in *T. prowazeki* resembles that described by Kirby (1944) in *T. termopsidis*. The new membrane approaches its full development rather late in division (pl. 16, *e-g*). However, after the daughter nuclei are formed it is impossible to distinguish between the parental and the daughter membranes.

It is apparent from the foregoing description that the posterior flagellum grows

out from the blepharoplast and attains a considerable length as a free flagellum. There are, however, two possible explanations for the development of the undulating membrane proper.

We might assume that the marginal filament and undulating membrane are direct derivatives of the posterior flagellum; if we should, our assumption would be largely in agreement with the opinion expressed by many students of trichomonads.

However, another interpretation may also be considered. It is possible that the marginal filament, which gives rise to the membranous sheath of cytoplasm, develops independently of the posterior flagellum, and that the developing membrane progressively incorporates the proximal part of the flagellum in the free margin. The frequent detachment of the posterior flagellum from the marginal filament, which may be observed in many trichomonadids, could be construed as support for this alternative.

Which of the two possibilities is correct may be ascertained only by further investigation of the morphogenesis of the undulating membrane in living organisms and in silver-impregnated preparations of many trichomonadids.

Axostyle and pelta.—The earlier and more recent literature concerning the reorganization of the axostyle was reviewed by Grassé (1926), Bishop (1931), Powell (1936), Samuels (1941), and Kirby (1944).

As pointed out by Kirby (1944), "most of the students of Trichomonadinae are now in agreement with the opinion that the old axostyle is dissolved and that new ones develop in connection with the blepharoplast and are entirely independent of the paradesmose." The present study of the morphogenesis of the axostyle in *T. prowazeki* lends further support to this contention.

The capitulum of the parental axostyle and also the old pelta show signs of degeneration quite early in division. However, little if any change may be observed prior to the time at which the two small daughter parabasal disks are formed (pl. 15, *a-c*).

In the early division stages that show the daughter parabasals, the capitulum of the axostyle and the pelta appear somewhat hypertrophied and displaced (pl. 15, *d, f*). Soon the capitulum, apparently together with the pelta, becomes definitely displaced in relation to the blepharoplasts (pls. 15, *h*; 16, *a*). Next, both of these structures disappear and only the trunk of the parental axostyle, first with (pl. 16, *b*) and then without (pl. 16, *c* and *d*) any remnant of the capitular expansion, is left in the cytoplasm. The trunk of the axostyle appears to degenerate quite rapidly. The degeneration proceeds from the anterior end toward the posterior end, and the old axostyle completely disappears prior to the time at which any indication of the constriction of the nucleus is observed (pl. 16, *e*). In this respect the behavior of the parental axostyle is unlike that of the parental axostyle in *Tritrichomonas muris* and in *T. augusta*, as described by Wenrich (1921) and Samuels (1941) respectively. In both of these species a remnant of the axostylar trunk could still be seen in late telophase. On the other hand, its behavior resembles closely that of the parental axostyle in *Caduceia monile*, in which, according to Kirby (1944), no trace of this structure is to be found in advanced anaphase or in later stages.

The earliest primordia of the new axostyles may be seen in dividing organisms in which a large remnant of the parental axostyle is still present (pl. 16, *a* and *b*).

Each primordium is represented by a short filament, or rod, originating in the blepharoplast (pl. 16, *a* and *b*). As division progresses the filament increases in length (pl. 16, *c*). Soon the pelta starts to develop in connection with the anterior part of the axostylar filament (pl. 16, *d*). Next, the filament is transformed into a lamella, which impregnates somewhat more deeply than the cytoplasm (pl. 16, *e*). At the same time the pelta becomes conspicuous (pl. 16, *e*). On the basis of the available preparations it is impossible to tell whether the expansion of the axostylar primordium involves the filament alone or whether the surrounding cytoplasm is also utilized in the process.

As division progresses the lamella and the pelta increase in size and in degree of differentiation (pls. 16, *g*; 17, *a*). Some time after the formation of the daughter nuclei the axostyle is clearly differentiated into the tubular trunk and the flattened, enlarged capitulum with the pelta applied to its anterior end (pl. 17, *b*). The posterior end of the trunk is typically blunt and not separated by a membrane from the surrounding cytoplasm (pl. 17, *b*). Further growth of the axostyle appears to involve little change in the size and shape of the capitulum and not much more in the width of the trunk (pl. 17, *c*); it seems to be concerned mainly with the increase in length of the trunk by the addition of material at the distal end, which remains unclosed even in far-advanced division stages.

The process of regeneration of the daughter axostyles resembles closely the homologous processes in *Tritrichomonas augusta* as described by Alexeieff (1924) and Samuels (1941), in *Trichomonas vaginalis* by Powell (1936), and in *Caduceia monile* by Kirby (1944).

Paradesmose.—Although the protargol impregnations are in general not as suitable for the study of the paradesmose as the hematoxylin-stained preparations, this structure may be seen with clarity in many dividing organisms treated with strong protein silver (pls. 16, *c*, *e*; 17, *a-c*). On some occasions the extranuclear spindle impregnates quite lightly (pl. 16, *c*, *e*), but on others rather heavily (pl. 17, *a* and *b*).

The width of the paradesmose appears to remain almost constant irrespective of its length (pls. 16, *e*; 17, *b* and *c*). Thus, as pointed out by Kirby (1944), "the elongation is apparently achieved by autonomous growth which in large part, at least, involves continuous addition to the substance of the paradesmose."

The presently available material was insufficient for tracing all the changes of the paradesmose during division of *T. prowazeki*. However, the stages in which it could be observed indicate that its behavior is not unlike that of the homologous structure in many other trichomonad species.

Double and multiple organisms.—Double and multiple organisms, in which nuclear division accompanied by full development of the mastigonts associated with the daughter nuclei is not followed by cytosomal division, have been reported by various writers in many trichomonadids and one monocercomonadid. An extensive review of the literature dealing with these multiple forms may be found in Bishop's (1931) paper. Kirby (1944) mentioned the fact that similar forms are occasionally encountered in many species of devescovichids.

During the present investigation flagellates with two or more mastigonts were encountered in the cultivated strains of *T. prowazeki*. The relative numbers of the

multiple forms varied among populations of a single strain. Furthermore, they were more abundant in some strains (for instance, from *Thamnophis elegans*) than in others (for instance, from *Rana catesbeiana*). Usually the organisms with fewer mastigonts, primarily double individuals, were more abundant.

Hinshaw (1926) found "duplex individuals" only in senescent cultures of *Trichomonas tenax*, but Bishop (1931) reported a lack of such correlation in her cultures of *Trichomonas* (= *Pentatrachomonas*) *hominis*. Cleveland (1928) could produce multiple organisms in *Tritrichomonas fecalis* by crowding the flagellates through centrifugation.

The double organisms were found in all strains irrespective of age. Although never very abundant, these forms are by far more numerous than any of the other multiple organisms. When observed in dark field they may appear quite similar to organisms in advanced stages of normal division, except that they are equipped with fully developed complements of anterior flagella, most frequently have the posterior projections of the axostyles, and remain together for a long time without ever showing any signs of cytosomal division.

On several occasions the populations of the culture-grown strains of *T. prowazeki* from the garter snakes contained organisms with three, four, five, six, or even eight mastigonts. The more mastigonts present the larger the cytosome. The largest forms are many times as large as the normal flagellates. When observed in dark field these enormous spheres exhibit a rotating movement. All the multiple forms observed were from cultures teeming with flagellates. Thus it seems possible that there is a correlation between crowded conditions and the production of multiple organisms in *T. prowazeki*.

Several multiple organisms were encountered in permanent preparations (pl. 17, *d* and *e*). In all these the mastigonts were fully developed and no sign of the parademesomere could be found.

The present investigation supports the view expressed by most protozoölogists that multiplication of mastigonts unaccompanied by cytosomal division occurs too erratically and relatively too infrequently to be considered part of the normal development of trichomonad flagellates.

TAXONOMIC SUMMARY

Trichomonas prowazeki Alexeieff

Trichomonas prowazeki Alexeieff, 1909, C. R. Soc. Biol. Paris, 67: 713.

Trichomonas (*Tetratrachomonas*) *prowazeki* Alexeieff, 1909; Parisi, 1910, Arch. Protistenk., 19: 232, pl. 14, figs. 1-5.

Tetratrachomonas prowazeki (Alexeieff, 1909), Alexeieff, 1911, C. R. Soc. Biol. Paris, 71: 540.

Tetratrachomonas batrachorum Escomé, 1925, Bull. Soc. Path. Exot., 18: 60, text fig. 5 on p. 59.

Trichomonas batrachorum Perty, 1852, pro parte, Bishop, 1931, Parasitology, 23: 137, pl. 6, figs. 1, 4.

"*Trichomonas* of the snake," Das Gupta, 1935, Jour. Trop. Med. London, 38: 151, pl. 2, figs. 1-8.

Type host.²—*Salamandra salamandra* (Linnaeus). Europe. (Alexeieff, 1909, 1911; Bishop, 1931.)

²In his original description of *T. prowazeki*, Alexeieff (1909) listed three amphibian hosts of this flagellate: *Salamandra maculosa* (= *salamandra*), *Triton cristatus*, and *Alytes obstetricans*. I have arbitrarily designated the first species on the list as the type host.

Additional hosts—

- Amphiuma means* Garden. Southern U.S.A. (Wenrich, 1947.)
Triton cristatus (Laurenti). Europe. (Alexeieff, 1909.)
Triturus torosus (Rathke). California. (Kofoid and Swezy, 1915.) Wild Cat Canyon, Contra Costa Co., California. Hyposyntype slides GP-135: 6, 7; GP-137: 11, 12.
Triturus viridescens (Rafinesque) [*Desmognathus torosus* Rafinesque?]. Eastern U.S.A. (vicinity of Baltimore, Maryland). (Tanabe, 1926.)
Alytes obstetricans (Laurenti). Europe. (Alexeieff, 1909, 1911.)
Bufo bufo (Linnaeus). England. (Bishop, 1931.)
Bufo terrestris (Bonnaterre). Mountain Lake Biological Station, Virginia. Hyposyntype slide GP-1221: 4.
Telmatobius jelskii (Peters) [*Telmatobius gebbsi* (Peters) ?]. Peru. (Escamel, 1925.)
Rana temporaria (Linnaeus). England. (Bishop, 1931.)
Rana boylei Baird. Reds Meadow, Madera Co., California. Hyposyntype slides GP-1226: 1, 3, 6, 7; GP-1227: 1, 3.
Rana catesbeiana Shaw. Eastern U.S.A. Hyposyntype slides GP-60: 10, 11; GP-133: 14, 17, 18.
Croodylus palustris (Lesson). India. (Aquarium in Milan.) (Parisi, 1910.)
Gekko vittatus Houttuyn. Paleliu, Palau Islands, West Pacific (San Diego Zoological Gardens). Hyposyntype slides GP-15: 2, 6.
Gehyra sp. Paleliu, Palau Islands, West Pacific (San Diego Zoological Gardens). Hyposyntype slides GP-14: 4; GP-107: 2, 5, 6; GP-108: 8, 9.
Natrix erythrogaster (Forster). U.S.A. (Das Gupta, 1935.)
Thamnophis elegans atratus (Kennicott). Dillon Beach, Marin Co., California. Hyposyntype slides GP-5: 1, 2; GP-23: 3, 4; GP-55: 3, 5, 8, 9, 10, 12.
Thamnophis ordinatus infernalis (Blainville). Dillon Beach, Marin Co., California. Hyposyntype slides GP-6: 2, 4, 6, 11, 13, 15, 17.

Diagnosis.—Body varying in shape, frequently elongate, body length $10.5(6.5-16.5)\mu$ in *Triturus torosus*, $10.5(6.5-15.5)\mu$ in *Rana boylei*, $10(7-22)\mu$ in *Rana catesbeiana*, $8.5(5.5-13)\mu$ in *Gehyra* sp., $12(6.5-21)\mu$ in *Thamnophis elegans atratus*, $10.5(6.5-22)\mu$ in *Thamnophis ordinatus infernalis*; body width $5.5(4.5-7.5)\mu$ in *Triturus torosus*, $6.5(4.5-10)\mu$ in *Rana boylei*, $6(4-18.5)\mu$ in *Rana catesbeiana*, $5.5(3.5-11)\mu$ in *Gehyra* sp., $7.5(4.5-15.5)\mu$ in *Thamnophis elegans atratus*, $6.5(4.5-18.5)\mu$ in *Thamnophis ordinatus infernalis*; four unequal to subequal anterior flagella, longest (fully grown) flagella $22.5(18.5-27.5)\mu$ in *Rana catesbeiana*, $20.5(18.5-24)\mu$ in *Thamnophis elegans atratus*; full complement of flagella typically restored prior to cytosomal division; slender costa and well-developed undulating membrane as long as body, with slight or no tendency to follow counterclockwise spiral course; free margin of undulating membrane ribbon-shaped, consists of inner filament and outer attached part of posterior flagellum; posterior flagellum of acroneme type, continues beyond end of undulating membrane for $10(7.5-13)\mu$ in *Rana catesbeiana* and *Thamnophis elegans atratus*; parabasal body typically circular disk with well-differentiated darkly impregnating margin and central granule, diameter about $1.75(1.5-2)\mu$ in *Rana catesbeiana*, about $2(1.5-2.5)\mu$ in *Thamnophis elegans atratus*, very close to blepharoplast, dorsal and to right of nucleus, rod-shaped in some views; "major" parabasal filament connects disk with blepharoplast, runs along dorsal edge of disk, continues posterior to disk for about $6.5-10\mu$ in *Rana catesbeiana* and *Thamnophis elegans atratus*; "minor" parabasal filament located at ventral border of disk, about $\frac{1}{4}-\frac{1}{2}$ of length of "major" filament; capitulum of axostyle spatulate, usually about three times as wide as trunk, constitutes anterior third part of axostyle, applied to ventral part of left surface of nucleus; trunk of axostyle about $0.3-0.4\mu$ in diameter, posterior projection $3.5(2-5)\mu$ long in *Rana catesbeiana*, $4(2-7.5)\mu$ long in *Thamnophis elegans atratus*, appears to taper gradually to point in most fixed preparations, abruptly and only in its terminal part in living material; pelta a long, crescent-shaped transverse membrane, ventral part of pelta applied to anterior segment of capitulum of axostyle, dorsal part tapers to point, often continues in long, slender filament; nucleus ellipsoidal or ovoidal $3(2-5.5)\mu \times 2(1.5-3.5)\mu$ in *Rana catesbeiana*, $3.25(2-4)\mu \times 2.25(1.5-3.5)\mu$ in *Thamnophis elegans atratus*, with long axis parallel or at small angle to long axis of cytosome.

There is no doubt that the size variations among different strains of a given flagellate symbiote and among different populations of a single strain depend to a large extent upon the environmental conditions prevailing in the host or in the culture. Indeed, by simply varying the amount of food in the culture medium or by changing the frequency of transfers we may greatly influence the size of the cultivated strain.

In fully grown organisms there are four usually unequal free flagella which originate in the anterior part of the blepharoplast complex (pls. 18, *d*; 19, *a-c*) and appear to be united in a short whip or column in their proximal part (pls. 18, *a, b, d*; 19, *a, c*). The anterior flagella end in rods or knobs (pl. 18, *b* and *c*). These terminal differentiations cannot be seen with clarity in heavily impregnated specimens (pl. 19, *a*) and do not show at all in preparations stained with iron hematoxylin or iron hematein (pl. 18, *a*). At times the flagella appear to be differentiated into two unequal groups, each of which contains two more or less subequal flagella (pl. 19, *a*). The inequality of the flagella, as well as their arrangement into two pairs, may be explained by the morphogenesis of these organelles. The longest full-grown flagella measured in twenty-five organisms in a single population of the strain from *Testudo* sp. were $19.5(15.5-23)\mu$ in length.

Flagellar counts based on 214 organisms of one population of the strain from *Testudo* sp. revealed 50 per cent of four-flagellate, and 34.5 per cent of three-flagellate individuals; in 15.5 per cent of the organisms the anterior flagella could not be counted. A similar count based on 100 organisms of another population of the same strain resulted in finding 55 per cent of four-flagellate, and 28 per cent of three-flagellate specimens; 18 per cent of the individuals were unsuitable for counting.

In both populations most of the three-flagellate organisms are somewhat smaller than the four-flagellate individuals. This finding correlates well with morphogenetic studies, according to which the three-flagellate forms are still immature.

The undulating membrane and the costa originate in the dorsal part of the blepharoplast complex (pls. 18, *d*; 19, *a-c*). The costa is a slender rod, which in its width approaches that of the flagella (pl. 18, *a-c*). The terminal segment of the rod tapers to a point (pl. 18, *b*).

The undulating membrane originates somewhat anterior to the costa (pls. 18, *d*; 19, *b*) and exhibits several bold undulations (pls. 18, *a-c*; 19, *a*). In more heavily impregnated organisms the free margin of the undulating membrane appears as a solid black ribbon (pls. 18, *d*; 19, *a-c*). In lighter, more favorable impregnations the ribbon may be seen to consist of two equally stout filaments (pl. 18, *b* and *c*). The outer filament represents the attached part of the posterior flagellum and continues beyond the posterior end of the undulating membrane as a free flagellum (pls. 18, *a-c*; 19, *a*). The free part of the posterior flagellum measured in twenty-five organisms of one population of the strain from *Testudo* sp. averaged in length $9(6.5-12)\mu$. The flagellum ends in an acroneme, which may be seen in some protargol-impregnated specimens (pl. 18, *b* and *c*) and in all living organisms observed in dark field.

The costa and the undulating membrane are as long as the cytosome, but since they invariably tend to follow a counterclockwise spiral course, neither of these

The parabasal apparatus is situated dorsally and to the right of the nucleus (pls. 18, *a-c*; 20, *a, e-h, n*). It consists of the two usual components: the parabasal body and the parabasal filament. The parabasal body is disk-shaped and usually exhibits a more or less pronounced dorsal flattening (pls. 18, *a-c*; 20, *c-n*). Whereas in some organisms it appears almost circular (pl. 20, *g, k*), in others its dorsal margin is greatly flattened (pls. 18, *c*; 20, *j, n*). Many of these differences in appearance are undoubtedly due to a large extent to the different positions of the disk; however, there may also be actual differences in shape. When viewed in some aspects the parabasal disk may appear as a stout, uniformly dark rod (pls. 19, *a*; 20, *a* and *b*). In most views, however, the disklike structure can be made out. Depending upon the intensity of impregnation, the disk varies in appearance from a uniformly dark body (pls. 18, *c*; 20, *i*) to an almost clear structure the center of which is occupied by a conspicuous dark granule (pls. 18, *b*; 20, *f, g, j, n*). Exceptionally, two granules are present (pl. 20, *k*). The longest axis of the parabasal disk (in the strain from *Testudo* sp.) ranges in length from approximately 1μ to about 1.5μ , averaging about 1.25μ .

The dorsal part of the disk is traversed by a rather stout parabasal filament (pls. 19, *a*; 20, *d, e, j-l, n*). This filament connects the parabasal body to the blepharoplast complex. Although the disk sometimes appears to be directly adjacent to the blepharoplast complex (pls. 18, *c*; 19, *a*; 20, *i, l, n*), it is more often removed from the blepharoplast complex by from less than 0.2μ (pl. 20, *g* and *h*) to more than 0.4μ (pl. 20, *e*). The space between the blepharoplast and the disk is traversed by the anterior segment of the parabasal filament (pl. 20, *e-h, j, k*). The filament continues for a considerable distance posterior to the disk (pls. 18, *b-d*; 19, *a, c*; 20, *a-n*). The free posterior part of the parabasal filament in the strain from *Testudo* sp. ranges in length from about 3.5μ to about 8.5μ , averaging about 5.25μ . This filament corresponds to the "major" parabasal filament of *Trichomonas prowazeki*. The "minor" filament, which could be seen with clarity in the latter species, was almost never observed in *T. brumpti*. A suggestion of this structure was encountered in a few organisms (pls. 19, *d*; 20, *m*), but as yet there is no adequate evidence for its presence.

Although the parabasal apparatus shows clearly in all protargol-impregnated organisms, it is but rarely visible in the material stained with iron hematein or iron hematoxylin. In a few organisms stained with iron hematein the parabasal apparatus can be made out, and in these organisms it appears as a faintly outlined disk (pl. 18, *a*); however, it never shows either the central granule or the parabasal filament.

The axostyle originates in the vicinity of the blepharoplast complex and appears to be connected to this granule (pls. 18, *b, d*; 19, *a-c*). The anterior third of the axostyle is expanded into a spatulate capitulum (pl. 19, *b*). In some organisms this expansion is slight (pl. 19, *a*), but in others it may be about three times as wide as the axostylar trunk (pl. 18, *b*). The capitulum is closely applied to the ventral part of the left surface of the nucleus (pl. 19, *a, c*). Often the ventral margin of the capitulum takes a heavy silver impregnation or hematoxylin stain, and in some views (pl. 18, *a, c*) the space between this margin and the ventral surface of the nucleus may be misinterpreted as a "cytostome." The latter structure has never been observed in either living or fixed and stained specimens of *T. brumpti*.

The trunk of the axostyle is a slender hyaline rod, which does not exceed about 0.2μ in width (pls. 18, *a-c*; 19, *a*) and shows neither axostylar granules nor periaxostylar rings. In undistorted organisms it is quite straight and appears to run through the center of the cytosome (pl. 18, *a* and *b*).

The terminal part of the axostyle projects from the posterior end of the cytosome. This projection, measured in twenty-five individuals of a single population of the strain from *Testudo* sp., averaged in length $3.75(2-5)\mu$. In fixed and stained preparations the distal segment of the projecting part seems to taper more (pls. 18, *c*; 19, *a*) or less (pl. 18, *a* and *b*) gradually to a point. In living organisms the tapering appears to be restricted to the very end of the axostyle, and is thus quite abrupt. Many protargol-treated specimens show a heavy impregnation of the axostylar projection (pls. 18, *c*; 19, *a*).

The pelta is a conspicuous crescent-shaped membrane anterior to the nucleus and close to the surface of the cytosome (pls. 18, *a-d*; 19, *a-d, f*). It is a rather stout structure (pls. 18, *d*; 19, *b*; 20, *a, b, d*), but in some views it may appear somewhat slenderer than in others (pl. 20, *c*). The ventral part of the pelta lies in close proximity to the anterior segment of the capitulum of the axostyle, to which it seems to be connected (pls. 18, *d*; 19, *b*). Although it is possible that the pelta represents a separate structure, there is a considerable amount of evidence that it actually constitutes a direct extension of the axostyle. The membrane runs dorsad and toward the right side (pls. 18, *b-d*; 19, *a-c, f*; 20, *a, e*). In its dorsal part it tapers gradually and finally ends in a slender filament of varying length (pls. 18, *c*; 19, *a, b, f*; 20, *d*). The pelta may be seen best in protargol-impregnated organisms, but it can also be observed in some of the preparations stained with iron hematein (pl. 18, *a*).

The nucleus is ovoidal (pls. 18, *c*; 20, *c, f, h, i, m, n*) or ellipsoidal (pls. 18, *a*; 19, *f*; 20, *e, g*) in shape. It is situated near the anterior end of the body with its longer axis parallel, or, more often, at a slight angle, to the anteroposterior axis of the cytosome. In twenty-five organisms of one population of the strain from *Testudo* sp. the nucleus ranged in length from 2μ to 4μ , in width from 1.5μ to 3.5μ , and averaged $3\mu \times 2.5\mu$. In preparations stained with iron hematein the resting nuclei show numerous fine granules and typically a single, large, spherical, dark-staining endosome, which is surrounded by a clear halo (pl. 18, *a*). At times two endosomes may be found.

The presence of bacteria (pl. 18, *a*) and of rice-starch grains in the cytoplasm indicates the ability of the organism to ingest solid food particles.

MORPHOGENESIS

The investigation of morphogenesis of *Trichomonas brumpti* was limited by the relatively small number of specimens in suitable stages of division in the available material. It was, consequently, less extensive than the comparable investigation of *Trichomonas prowazeki*.

As far as could be determined, the morphogenetic processes of *T. brumpti* are, in most respects, similar to those of *T. prowazeki*. This basic similarity may be ascertained by a comparison of the figures depicting various division stages of the former species (pls. 19, *d* and *e*; 20, *o-q*; 21, *a* and *b*) with the more numerous and more

complete representations of division stages of the latter (pls. 15–17). Thus the previously given, detailed description of morphogenesis of *T. prowazeki* may in most respects also apply to that of *T. brumpti*.

However, there appear to be certain differences in the life cycle of the two trichomonads. These differences are mainly concerned with the time of appearance of the third and fourth anterior flagella. Consequently, it seems necessary to give here a brief account of the steps involved in the restoration of the full complement of anterior flagella in *T. brumpti*.

The primordium of the third flagellum does not appear at either of the poles until rather late in division. The short stumps of these flagella may be first seen in division stages which exhibit two daughter nuclei (pl. 21, *a* and *b*). However, some of the dividing organisms with two nuclei still possess only two flagella at the poles (pl. 20, *q*).

The fourth flagellum was not observed in even very advanced stages of division (pl. 21, *b*). On the other hand, there are at times as many as 34.5 per cent of three-flagellate solitary individuals present in a single population (pl. 19, *f*). The size of these organisms falls in most cases below the average size of the population to which they belong. In some four-flagellate individuals one of the anterior flagella may be quite short (pl. 21, *e*). The smaller double individuals exhibit only three anterior flagella in each of the two otherwise fully developed mastigonts (pl. 21, *c*), whereas the larger ones have four flagella in each mastigont (pl. 21, *d*).

The afore-mentioned facts seem to indicate clearly that the full complement of anterior flagella in *T. brumpti* is restored after cytosomal division has been completed.

In many four-flagellate individuals the fourth flagellum is almost as long as the third (pl. 18, *c*; 19, *a*), and this suggests that the rate of growth of the former may exceed that of the latter. On the other hand, division may start in some of the organisms which still exhibit a short fourth flagellum (pl. 20, *o*). Usually the two new flagella never reach the full length of the parental ones (pls. 18, *c*; 19, *a*) and may often be easily recognized even in presumably mature individuals (pl. 19, *a*).

TAXONOMIC SUMMARY

Trichomonas brumpti Alexeieff

Trichomonas (Tetratrichomonas) brumpti (*T. prowazeki* Alex. pro parte) Alexeieff, 1912, Zool. Anz., 40: 98, text fig. 1: 1, 2, on p. 99.

Trichomonas brumpti Alexeieff, 1912, Wenyon, 1926, Protozoology (2 vols.; New York, William Wood and Co.), 1: 669.

Type host.—*Melanochelys trijuga* (Schweigger). Ceylon. (Alexeieff, 1912.)

Additional hosts—

Testudo calcarata (= *Testudo sulcata* Miller) Schneider. Sudan. (Wenyon, 1926.)

Testudo (argentina) chilensis Gray. South America (London Zoölogical Gardens). (Wenyon, 1926.)

Testudo radiata Shaw. Madagascar (London Zoölogical Gardens). (Wenyon, 1926.)

Testudo hoodensis Van Denburgh. Hood Island, Galápagos Archipelago (San Diego Zoölogical Gardens). Hyposyntyple slides GP-41: 3, 4.

Testudo sp. Galápagos Archipelago (San Diego Zoölogical Gardens). Hyposyntyple slides GP-19: 5, 9; GP-56: 1, 3, 4; GP-64: 4, 5, 6.

Gopherus agassizii (Cooper). South California desert (San Diego Zoölogical Gardens). Hyposyntyple slides GP-71: 4, 5.

Diagnosis.^a—Body stout, ellipsoidal or ovoidal; length $9(5.5-13)\mu$ in *Testudo* sp., $7(5.5-8)\mu$ in *Testudo hoodensis*, $6.5(5.5-7.5)\mu$ in *Gopherus agassizii*; width $7.25(4.5-11)\mu$ in *Testudo* sp., $5.5(4.5-6)\mu$ in *Testudo hoodensis*, $4.5(3.5-6)\mu$ in *Gopherus agassizii*; four usually unequal anterior flagella, fully grown flagella $19.5(15.5-23)\mu$; full complement of anterior flagella restored after cytoplasmic division, thus up to 34.5 per cent of three-flagellate immature forms in a population; costa and undulating membrane counterclockwise spiral, as long as body; costa slender; free margin of well-developed undulating membrane ribbon-shaped, consists of inner filament and outer attached segment of posterior flagellum; posterior flagellum continues beyond end of undulating membrane for $9(6.5-12)\mu$, terminates in acroneme; parabasal body dorsally flattened disk situated dorsal and to right of nucleus, longest axis about $1.25(1-1.5)\mu$; parabasal filament connects disk with blepharoplast complex, continues posterior to disk for about $5.25(3.5-8.5)\mu$; capitulum of axostyle spatulate, from less than 2 to 3 times as wide as trunk, applied to ventral part of left surface of nucleus; trunk of axostyle slender hyaline rod, about 0.2μ in diameter, posterior projection $3.75(2-5)\mu$ long, tapers to a point in terminal segment; pelta well-developed transverse crescent-shaped membrane anterior to nucleus, ventral part closely applied to anterior segment of axostylar capitulum, dorsal part tapers to a point prolonged in fine filament; nucleus ellipsoidal or ovoidal, $3(2-4)\mu \times 2.5(1.5-3.5)\mu$; longer axis of nucleus parallel or at slight angle to anteroposterior axis of cytosome.

COMPARISON OF TRICHOMONAS PROWAZEKI AND TRICHOMONAS BRUMPTI

Whereas *Trichomonas prowazeki* appears to be widely distributed among amphibian and reptilian hosts, *T. brumpti* is apparently restricted to the chelonians and has been hitherto found only in the members of the family Testudinidae (land tortoises and terrapins) of that order.

There are many morphological similarities between *T. prowazeki* and *T. brumpti*. These consist in the unequal or subequal flagella, the appearance of the costa and of the undulating membrane, the structure of the parabasal apparatus, and the general character of the axostyle and of the pelta.

On the other hand, there are also certain more or less pronounced differences between the two species in question. *Trichomonas prowazeki* appears to be typically slenderer than *T. brumpti*. The average length of the former species is somewhat greater than that of the latter. The course of the costa and of the undulating membrane is a pronounced counterclockwise spiral in the flagellate from chelonians. Although the degree of the turn is never as great as that exhibited by the homologous organelles in *Tritrichomonas muris* (see Kirby and Honigberg, 1949a), it is sufficient to prevent them from reaching the posterior end of the cytosome. In contrast, the costa and the undulating membrane in *T. prowazeki* show either little or no tendency to follow a spiral course.

The parabasal body of *T. prowazeki*, although it may exhibit at times some degree of dorsal flattening, is typically a uniform, circular disk. The disk is relatively thick and has a conspicuous, darkly impregnating margin. It is situated very close to the blepharoplast and often appears to be in direct contact with this granule. The "major" parabasal filament is very long and may almost reach the posterior end of the cytosome. The "minor" parabasal filament is clearly visible in most organisms. Although the parabasal apparatus of *T. brumpti* is generally quite similar to that of *T. prowazeki*, it has a marked tendency toward dorsal flattening,

^a All measurements of mastigont organelles were taken in one population of the strain from *Testudo* sp.

appears to be considerably thinner, and never exhibits a dark margin. Although the disk is situated quite close to the blepharoplast, it is usually separated from the granule by a narrow free space traversed by the anteriormost part of the "major" parabasal filament. The "major" parabasal filament is somewhat shorter in *T. brumpti* than in *T. prowazeki*. The "minor" parabasal filament is very difficult to demonstrate in the former species, and it is impossible to be sure whether it actually exists in most of the organisms.

The capitulum of the axostyle appears to be relatively narrower and less well differentiated in the species from chelonians, and the axostylar trunk is considerably thinner. On the other hand, the pelta of *T. prowazeki* is somewhat longer and slenderer, and its dorsal part is often continued into a very long, fine filament.

In addition to the morphological differences there is a certain amount of difference in the morphogenesis of the two organisms. The third anterior flagellum appears to originate somewhat earlier, and the fourth flagellum considerably earlier, in division of *T. prowazeki* than in that of *T. brumpti*. Since the full complement of anterior flagella is restored prior to cytosomal division in the former species and after cytokinesis in the latter species, the proportion of three-flagellate solitary organisms, almost negligible in *T. prowazeki*, may be quite high in *T. brumpti*. In addition, the inequality of the anterior flagella is more pronounced in the species from chelonians.

COMPARISON WITH OTHER SPECIES OF THE GENUS *TRICHOMONAS* DONNÉ

Of all the species of the four-flagellate genus *Trichomonas* Donné, 1836, only a few have been studied in protargol-treated preparations and can thus be fairly compared with those described in the present paper.

Two of these species, *Trichomonas termopsidis* Cleveland and *Trichomonas limacis* Dujardin, studied by Kirby (1944) and Kozloff (1945) respectively, occur in invertebrate hosts, the former in termites and the latter in gastropod mollusks. Besides the profound differences in hosts, the two afore-mentioned trichomonads exhibit striking morphological differences from either *T. prowazeki* or *T. brumpti*. *T. termopsidis* is usually much larger and has a very long, often twisted or coiled, somewhat flattened, and at times branched parabasal body which encloses a row of granules. Its axostyle, which contains numerous granules, is very stout, and the pelta is absent. Although *T. limacis* is more similar to the presently described species in many details of structure, it is somewhat larger, has a U- or V-shaped, long-armed parabasal apparatus and a very slender pelta.

Silver impregnations of *Trichomonas vaginalis* Donné and *Trichomonas tenax* (O. F. Müller) indicate that these symbiotes of the human vagina and mouth respectively differ from *T. prowazeki* and *T. brumpti* not only in the length of the costae and undulating membranes, which are never as long as the cytosome in either of the former two species, but also in the structure of the parabasal apparatus. The parabasal body appears to be V-shaped in *T. vaginalis* and rod-shaped in *T. tenax*.

The remaining species studied in protargol, *Trichomonas microti* Wenrich and Saxe, from rodents (see Wenrich and Saxe, 1948, 1950), and *Trichomonas guttula*

Kirby and Honigberg, from marsupials (see Kirby and Honigberg, 1949b, 1950), are quite similar to *T. brumpti* and *T. prowazeki*. However, both *T. microti* and *T. guttula* occur in warm-blooded hosts and show certain morphological differences from the congeneric symbiotes of amphibians and reptiles. *T. microti* has a smaller average size than all strains of *T. prowazeki* and some strains of *T. brumpti*. In the length and amount of development of the undulating membrane, *T. microti* and *T. brumpti* are quite similar. The costa, though of similar relative length in these two species, is slenderer in the flagellate from chelonians. Both of the afore-mentioned organelles seem to follow a more sharply spiral course in the trichomonad from rodents. The axostyle of *T. brumpti* is much thinner than that of *T. microti* and does not taper abruptly immediately upon emerging from the body. The pelta of *T. brumpti* is quite similar to that of *T. microti*. The differences between *T. microti* and *T. prowazeki* are not unlike those between *T. brumpti* and *T. prowazeki*, except that in the width of the trunk of the axostyle *T. microti* more closely resembles *T. prowazeki*. The most striking difference between the two species from the cold-blooded hosts and *T. microti* is in the structure of the parabasal apparatus. This organelle is discoid in *T. prowazeki* and *T. brumpti* and Y-shaped in the trichomonad from rodents. Although nothing is known about the morphogenesis of *T. microti* (Wenrich and Saxe, 1950, did not mention the presence of three-flagellate individuals in this species, and all the figures included in their paper depict organisms with four well-developed anterior flagella), in the organisms represented in figures 11, 15, 18 on page 269 in Wenrich and Saxe's paper the anterior flagella may be differentiated into two groups. The flagella of one of the groups are longer than those of the other, and each group consists of two slightly subequal flagella. It is thus not unlikely that this situation, which in some respects resembles the one reported by Alexeieff (1909) and seen in *T. prowazeki* by me, is the result of a similar development of the anterior flagella in the latter species and in *T. microti*.

In most structural details *T. guttula* resembles *T. brumpti*. The shape and size of the body, the development of the anterior flagella, the appearance of the undulating membrane and of the costa, the size and shape of the parabasal body and of the parabasal filament, the width of the trunk of the axostyle, and the type and size of the projecting part of the axostyle as well as of the pelta are closely comparable in the two species. However, the capitulum of the axostyle is usually more conspicuous and the spiral of the undulating membrane somewhat less pronounced in the flagellate from marsupials. Furthermore, its parabasal disk exhibits more striking dorsal flattening and often looks like a partly formed drop hanging from the parabasal filament. The disk was never observed to be as round as the comparable structure in many representatives of *T. brumpti*. Thus the four-flagellate trichomonadids from chelonians and marsupials are quite similar in their structure, yet the slight differences, which may be brought out by a careful comparison, and the fact that the symbiotes occur in hosts which, from the taxonomic and physiological viewpoints, are very different from each other, preclude the placing of *T. brumpti* and *T. guttula* in a single species. All the differences existing between *T. prowazeki* and the congeneric trichomonad from chelonians are also found to separate the former species from *T. guttula*.

COMMENTS ON THE DISCOID PARABASAL BODY AND NUMBER OF ANTERIOR FLAGELLA IN TRICHOMONAD FLAGELLATES

The parabasal apparatus consisting of a disk and often of one or two filaments is not restricted to *Trichomonas guttula*, *T. prowazeki*, and *T. brumpti* or to the members of the genus *Trichomonas* Donné in general. Similar parabasals have been reported in *Pentatrichomonas hominis* by Kirby and Honigberg (1949b, 1950) and by Wenrich and Saxe (1948, 1950), in *Tricercomitus termopsidis* and *T. divergens* by Kirby (1930), in *Pentatrichomonoides scroa* by Kirby (1931), in *Trimitus parvus* by Grassé (1932), and in *Monocercomonas caviae*, *M. pistillum*, *M. minuta*, and *Hexamastix caviae* by Nie (1950).

Pentatrichomonas hominis possesses a very small disk or granule of parabasal material (less than 1μ in diameter) which is applied to the filament somewhat posterior to the blepharoplast complex. In the two species of *Tricercomitus* from termites the parabasal is, according to Kirby (1930), "drop-shaped or elongate in form, and of diameter and length somewhat less than that of the nucleus." No filament was seen to be associated with the body. A similar parabasal apparatus was figured by Grassé (1932) in *Trimitus parvus*. Kirby's (1931) figures of *Pentatrichomonoides scroa* (figs. 66, 69, and 70 on his pl. 20) represent a parabasal apparatus which in many respects resembles that of *T. prowazeki* or *T. brumpti*.

Nie (1950) described a "ring-shaped" parabasal body in three species of *Monocercomonas* and in one of *Hexamastix* from the guinea pig, *Cavia porcella*. He noted that in *Monocercomonas caviae* the ellipsoidal parabasal appeared solid black in intense impregnations and assumed the "hollow-ring" appearance in well-washed specimens. It is probable that a similar situation was observed, although unmentioned, by Nie in the remaining trichomonads which showed "ring-shaped" parabasals (see pl. 6, figs. 124, 132, in his paper). Thus it seems likely that he dealt with disk-shaped organelles, the differentiated margin of which showed a higher affinity for protargol. Although the disks apparently lack the central granule, they certainly resemble the parabasal body of *Trichomonas prowazeki*.

In *Hexamastix caviae* Nie found two equal filaments associated with the "ring." Thus the parabasal body of the six-flagellate monocercomonad approaches in most details the homologous apparatus I encountered in *T. prowazeki*. However, in the latter species the filaments are definitely unequal in both length and width. My own observations of *Monocercomonas colubrorum* from various squamate reptiles revealed in this species a parabasal apparatus not unlike that described by Nie (1950) in the congeneric flagellates from the guinea pig. There is no doubt that further investigation will bring out similar parabasal structures in many other trichomonads.

The study of the development of the full complement of anterior flagella in *Trichomonas guttula* by Kirby and Honigberg (1950) and the present study of the same process in *Trichomonas prowazeki* and *T. brumpti* provide an additional explanation for the variation in the number of anterior flagella in populations of otherwise morphologically identical trichomonad flagellates.

Many examples could be cited in which the number of anterior flagella was

reported to vary in different trichomonad species. Some of the records may be found in the works by Duboscq and Grassé (1923, 1924a, b), Kirby (1930), Bishop (1931), Oguma (1931), Grassé (1932), Callender and Simmons (1936), Wenrich (1944a, b), and Nie (1950).

There is no doubt that the flagella, which could not be clearly demonstrated by most of the commonly employed techniques, were sometimes merely miscounted. At other times some of the flagella were probably lost in the preparation of the permanent smears. Undoubtedly most, if not all, of the records of two-flagellate solitary forms of trichomonads (for instance, Cutler, 1919) were affected by one of these two errors.

Still other reports of variation in the number of anterior flagella probably dealt with two morphologically distinct species. Such undoubtedly was the case with "*Trichomonas batrachorum*," which, according to Bishop's (1931) description, included three- and four-flagellate forms present in equal numbers in cultures. As pointed out elsewhere in this paper, she worked probably with mixed cultures of *Tritrichomonas batrachorum* and *Trichomonas prowazeki*.

In the same paper Bishop mentioned the presence of rare five-flagellate individuals in her cultures of "*Trichomonas batrachorum*." Wenrich (1944b) found a few five-flagellate organisms in populations of *Trichomonas vaginalis* and *T. tenax*. It is possible that these and other authors who reported supernumerary anterior flagella in trichomonads could have actually seen very early division stages marked by the appearance of the new posterior flagellum.

As shown by Kirby and Honigberg (1950) and by the present study, in at least two trichomonad species the morphogenetic process involved in the restoration of the full flagellar complement may be responsible for the appearance of three-flagellate young individuals together with four-flagellate mature organisms. The description of variations in the number of anterior flagella in *Pentatrichomonas hominis* by Wenrich (1944a) suggests that the differences may possibly be explained on a similar basis. Kirby (1930) mentioned profound variations in the number of anterior flagella in *Hexamastix termopsidis*, and Nie (1950) reported similar variations in *H. caviae* and *H. robustus* from the guinea pig. Since they did not observe the behavior of the flagella in division, it is impossible to know whether, in the case of these monocercomonads, an explanation of the variation could be derived from a morphogenetic study. Kirby's (1930) statement, "It seems that the completed number [of anterior flagella] is five, that these separate three and two in division, and that outgrowth to completion takes place slowly, and one flagellum at a time," appears to give some support to such an assumption.

Since many known and perhaps as many unknown factors, both natural and artificial, bring about differences in the number of the anterior flagella observed in specimens of a given trichomonad species, the acceptance of the idea that the number of flagella may vary in fully grown representatives of a single species would have to be based on sound technical procedures and on a very careful analysis of the structure and morphogenesis of many populations.

At present a great deal of evidence has been and is still being accumulated in support of the contention that in trichomonad flagellates, as in many other mastigote groups, the number of flagella is constant in fully developed members of each species.

SUMMARY

Trichomonas prowazeki Alexeieff was found among amphibians in *Triturus torosus*, *Bufo terrestris*, *Rana boylei*, and *Rana catesbeiana*; among reptiles it was observed in *Gekko vittatus*, *Gehyra* sp., *Thamnophis elegans atratus*, and *Thamnophis ordinatus infernalis*. All these findings, except for *Triturus*, constitute new host records.

Trichomonas prowazeki Alexeieff is characterized by four unequal or subequal flagella. Its slender costa and well-developed undulating membrane exhibit little tendency to follow a counterclockwise spiral course. The marginal flagellum continues beyond the posterior end of the membrane and ends in an acroneme. The parabasal apparatus consists of a rather thick circular disk with a dark margin and a central granule, as well as of two parabasal filaments. The capitulum of the axostyle is spatulate, and the trunk of the axostyle is of a medium caliber. The well-developed, crescent-shaped pelta is usually slender. Its ventral part is closely applied to the anterior segment of the axostylar capitulum, of which the pelta is probably a direct extension. Feeding may often be accomplished by formation of filamentous cytoplasmic extensions in the region adjacent to the area at which the axostyle projects from the cytosome. These extensions carry food into the posterior part of the body.

In division the four parental flagella are equally distributed between the two daughter individuals. The third flagella originate at about the time of the formation of the daughter nuclei, and the fourth flagella appear still later, but typically prior to cytosomal division. The old parabasal apparatus appears to be almost completely discarded, and two are neofomed very early in division. The parental costa and undulating membrane are retained by one of the daughter individuals; the other individual develops a new complement of homologous organelles. There is evidence that the development of the posterior flagellum precedes somewhat that of the undulating membrane. Either the marginal filament and the cytoplasmic sheath are direct derivatives of the posterior flagellum, or the former two components develop independently and become united with the flagellum during division. The parental axostyle and pelta are completely discarded and new ones are formed. The cytosomal division is a modification of the longitudinal type.

Trichomonas brumpti was encountered in *Testudo hoodensis*, in *Testudo* sp., and in *Gopherus agassizii*. All these findings constitute new host records.

In many respects *T. brumpti* is similar to *T. prowazeki*. However, the species from chelonians is usually smaller and stouter, its costa and undulating membrane follow a pronounced counterclockwise spiral course, its axostyle is very slender, and its pelta rather stout. The parabasal body of *T. brumpti* is quite similar to that of *T. prowazeki*; however, it is thinner, exhibits a pronounced dorsal flattening, lacks a dark margin, and is typically accompanied by only one filament.

The morphogenesis of *T. brumpti* resembles that of *T. prowazeki*, but the third flagellum originates somewhat later, and the fourth does not appear until after the cytosomal division.

A comparison of *T. prowazeki* and *T. brumpti* with other members of the four-flagellate genus *Trichomonas* (which have been studied in protargol-treated prepa-

rations) reveals that they most resemble *T. microti* Wenrich and Saxe and *T. guttula* Kirby and Honigberg. However, these two species occur in warm-blooded hosts, and may be differentiated from the ones described in this paper on morphological grounds.

Flagellates with a discoid parabasal apparatus are found in at least three species belonging to the genus *Trichomonas*, as well as in some species of other trichomonad genera. Although there exist certain differences among these parabasals found in different flagellate species, the basic pattern remains the same.

A careful examination of the structure and morphogenesis of many trichomonads seems to support the contention that in this group the number of flagella is constant in fully developed members of each species.

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PLATES

All figures have been made with the aid of camera lucida.

Abbreviations for methods of preparation: Holl., Hollande's cupric picroformol; Hematein, mordant in 0.5 per cent alcoholic solution of iron alum, and stain in 0.67 per cent alcoholic solution of hematein; Prot., impregnation by strong protein silver, protargol.

PLATE 13

Trichomonas prowaseki Alexeieff

a. From *Thamnophis ordinatus infernalis*. Composite diagram showing all mastigont organelles. Pelta stained rather lightly; parabasal body represented by a disk which fails to show either dark margin or central granule; no parabasal filaments show; nucleus contains numerous small chromatic granules and large peripheral endosome surrounded by clear halo; many dark-staining inclusions present in cytoplasm. Actual length of cytosome, 12 μ . Holl. Hematein. $\times 3,930$.

b, c. From, respectively, *Thamnophis elegans atratus* and *Banacatesberiana*. Right views showing all mastigont organelles. In *b*, note the long filamentous dorsal extension of the pelta. Parabasal body more typical in shape in *c*, but no "minor" parabasal filament associated with it. Actual length of cytosome in *b*, 11.5 μ . Holl. Prot. *b*, $\times 3,870$; *c*, $\times 3,930$.

d. From *Thamnophis elegans atratus*. Apical view showing relationship of mastigont organelles. Parabasal body optically telescoped and appears as a stout rod. Holl. Prot. $\times 3,930$.

e-g. From *Thamnophis elegans atratus*. Diagrams (side views) showing various modifications in structure of the parabasal body in nondividing flagellates. Holl. Prot. $\times 3,870$.

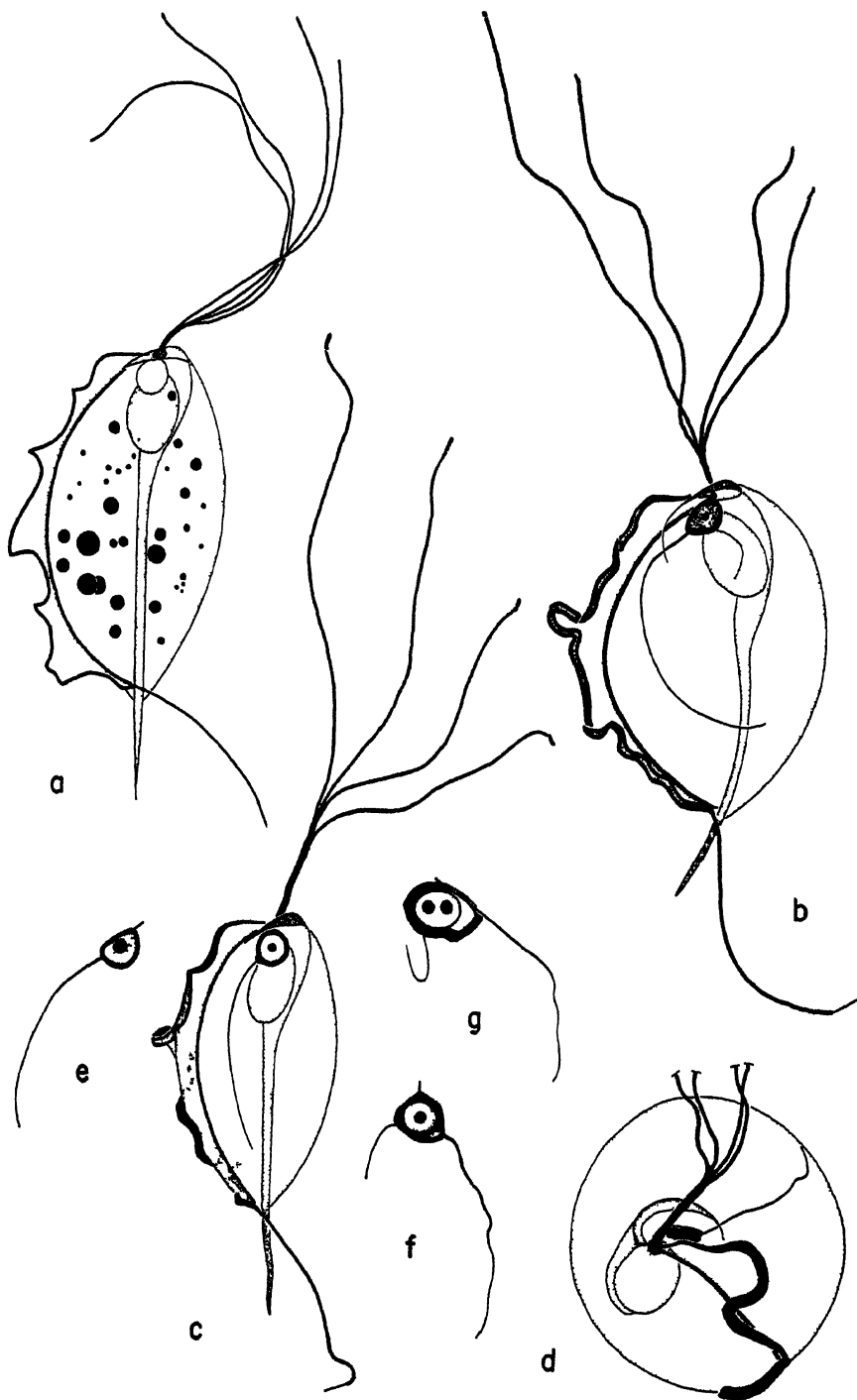


PLATE 14

Trichomonas prowazeki Alexeieff
from *Thamnophis elegans atratus*. Holl. Piot.

a. Right view, showing all mastigont organelles. Note the typical uniformly circular, disk-shaped parabasal body with dark margin and central granule, and "major" and "minor" parabasal filaments associated with dorsal and central parts of the disk respectively. Actual length of cytosome, 12μ . $\times 3,930$.

b. Right view of a large nondividing flagellate. Pelta and parabasal apparatus enlarged; parabasal body shows two central granules and median constriction. A small organism of the same species may be seen in the cytosome of the larger one. $\times 3,870$.

c, d. Diagrams of mastigont structures in left aspect, showing relationship between spatulate capitulum of axostyle and pelta. Parabasal apparatus omitted. $\times 3,870$.

e. Ventral view of anterior part of cytosome, showing all mastigont structures. Dorsal tapering part of pelta turns toward left and continues in a long filament; parabasal body appears as uniformly wide rod with double margin. Actual width of cytosome, 11μ . $\times 3,870$.

f. Dorsal view of anterior part of cytosome, showing most mastigont structures. Parabasal body appears as uniformly wide rod with double margin. Costa and undulating membrane omitted. $\times 3,870$.

g. Diagram of pelta, capitulum of axostyle, and enlarged parabasal apparatus of a large nondividing flagellate. The pelta appears to be a direct extension of the axostyle. $\times 3,870$.

h. Diagram of parabasal apparatus in anterodorsal view. $\times 3,870$.

i, j. Diagrams of enlarged parabasal apparatus. The parabasal bodies show two central granules. $\times 3,870$.

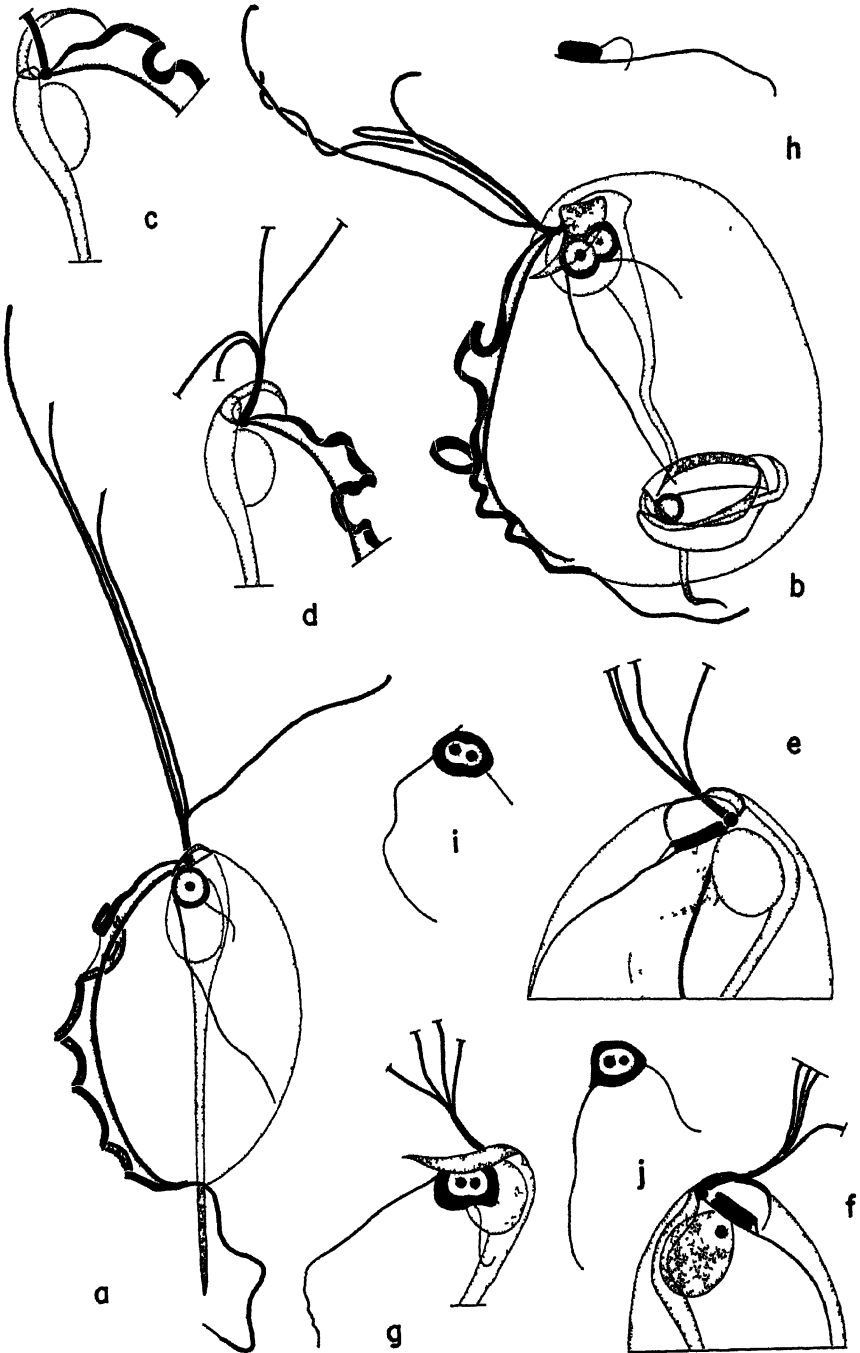


PLATE 15

Trichomonas prowazeki Alexeieff
from *Thamnophis elegans atratus*. Holl. Prot.
Division

a, b. Early stages. Old detached parabasal apparatus is lodged some distance posterior to blepharoplast. In *a*, the stout fibril between the parabasal discoid and blepharoplast probably consists of new costa and parabasal filament. In *b*, the two structures are separate. No parabasal material is seen at blepharoplast. Actual length of cytosome in *a*, 10μ . $\times 3,930$.

c. Stage similar to the ones in *a* and *b*. Distal segments of new costa and parabasal filament are separate. The longer and more peripheral filament represents the new costa. A small amount of probably parental material is at the blepharoplast. Old undulating membrane not shown. $\times 3,930$.

d-f. Stages somewhat later than those in *a-c*, showing two new parabasals. In *d*, new costa and "major" parabasal filament which is associated with dorsal disk remain in close spatial relationship; in *e*, the two structures are largely separate; in *f*, new costa is already quite stout. In *d*, the axostylar capitulum shows early signs of degeneration and is somewhat displaced in relation to blepharoplast. In *f*, capitulum and pelta show still more degeneration (hypertrophy). Actual width of cytosome in *f*, 7μ . *d*, e , $\times 3,930$; *f*, $\times 3,870$.

g. Diagram of a division stage corresponding to the ones in *d-f*. Most organelles not shown. One of the two new parabasal disks is enlarged and exhibits two central granules. $\times 3,930$.

h. A more advanced stage. Daughter blepharoplasts some distance apart; two flagella associated with each; capitulum of old axostyle shows degenerative hypertrophy and is displaced in relation to blepharoplast; parabasal structures well developed; new costa quite conspicuous. New posterior flagellum and undulating membrane fail to show. Actual length of cytosome, 17μ . $\times 2,650$.

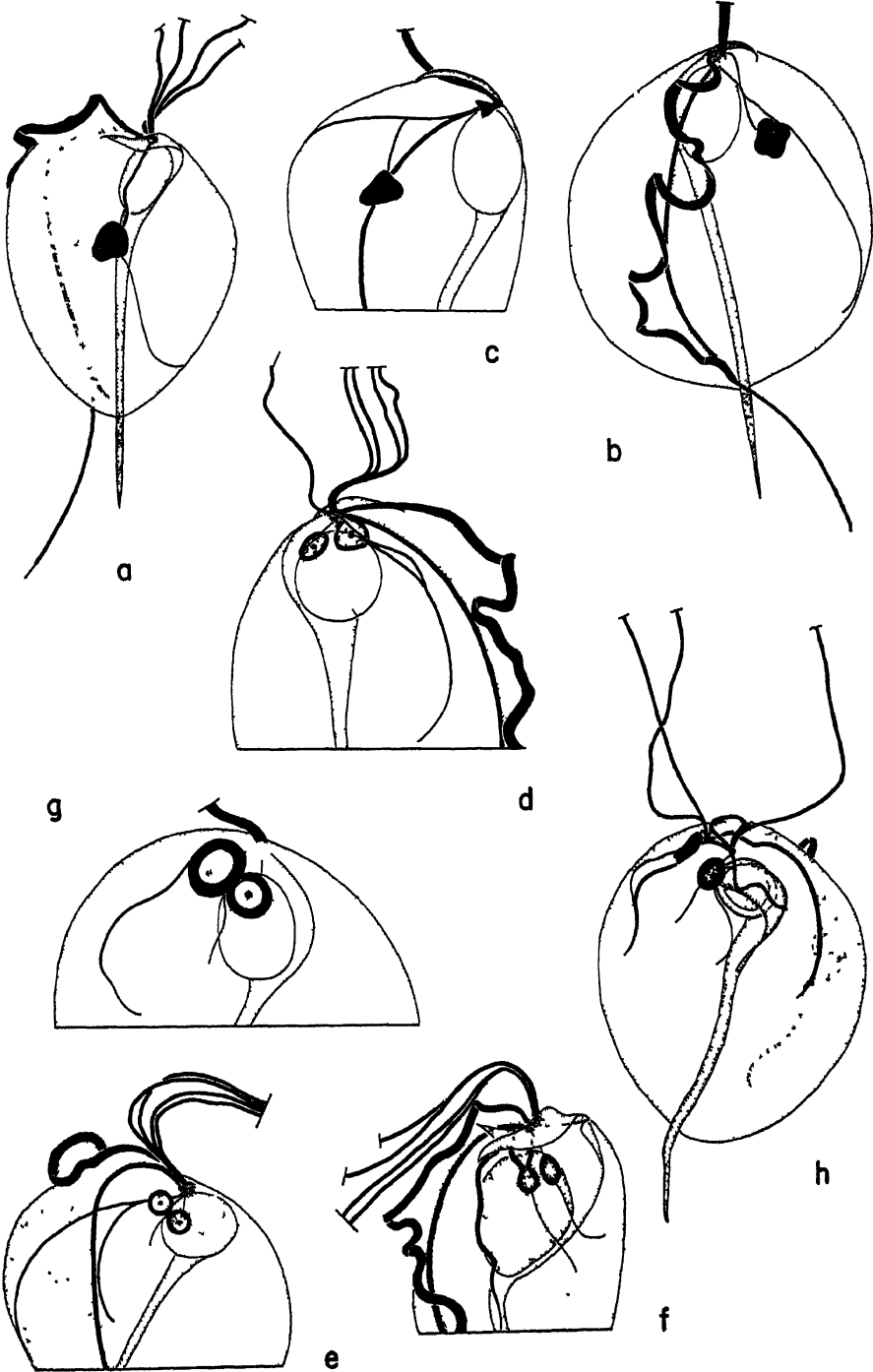


PLATE 16

Trichomonas prowaziki Alexeieff

from *Thamnophis elegans atratus*. Holl. Prot. $\times 2,650$

DIVISION

a. Daughter blepharoplasts somewhat farther apart than in plate 15, *h*; "major" parabasal filament associated with one of the parabasal bodies is quite long; new costa well developed; new posterior flagellum has reached a considerable length; developing undulating membrane associated with proximal segment of the flagellum. Capitulum of parental axostyle shows degenerative hypertrophy; minute primordium of new axostyle seen at pole to observer's right. Actual length of cytosome, 14.5μ .

b. Costa and new posterior flagellum longer than those in *a*; developing undulating membrane associated with proximal segment of the flagellum; part of axostylar capitulum has already disappeared; primordium of new axostyle seen with clarity at pole to observer's left.

c. New undulating membrane associated with large segment of the posterior flagellum; only trunk of parental axostyle still persists in cytosome; paradesmose connects daughter blepharoplasts.

d. Stage similar to that in *c*. Margin of new undulating membrane distinctly double; sheath of protoplasm along inner marginal filament; distal segment of marginal filament of parental undulating membrane is torn off the posterior flagellum; primordium of new pelta at upper pole.

e. Daughter blepharoplasts connected by paradesmose are quite far apart; new undulating membrane well developed and attached to costa; posterior segment of its marginal filament separated from posterior flagellum; axostylar primordium at pole to observer's right appears as a lamella; new peltas visible at both poles; parental axostyle no longer present.

f, g. Nucleus in late telophase. In *g*, it is just about to separate into two daughter nuclei. New costae and undulating membranes almost fully developed. New axostyles appear as filaments in *f*, but are lamellate in *g*. In *g*, well-developed pelta is seen at pole to observer's right.

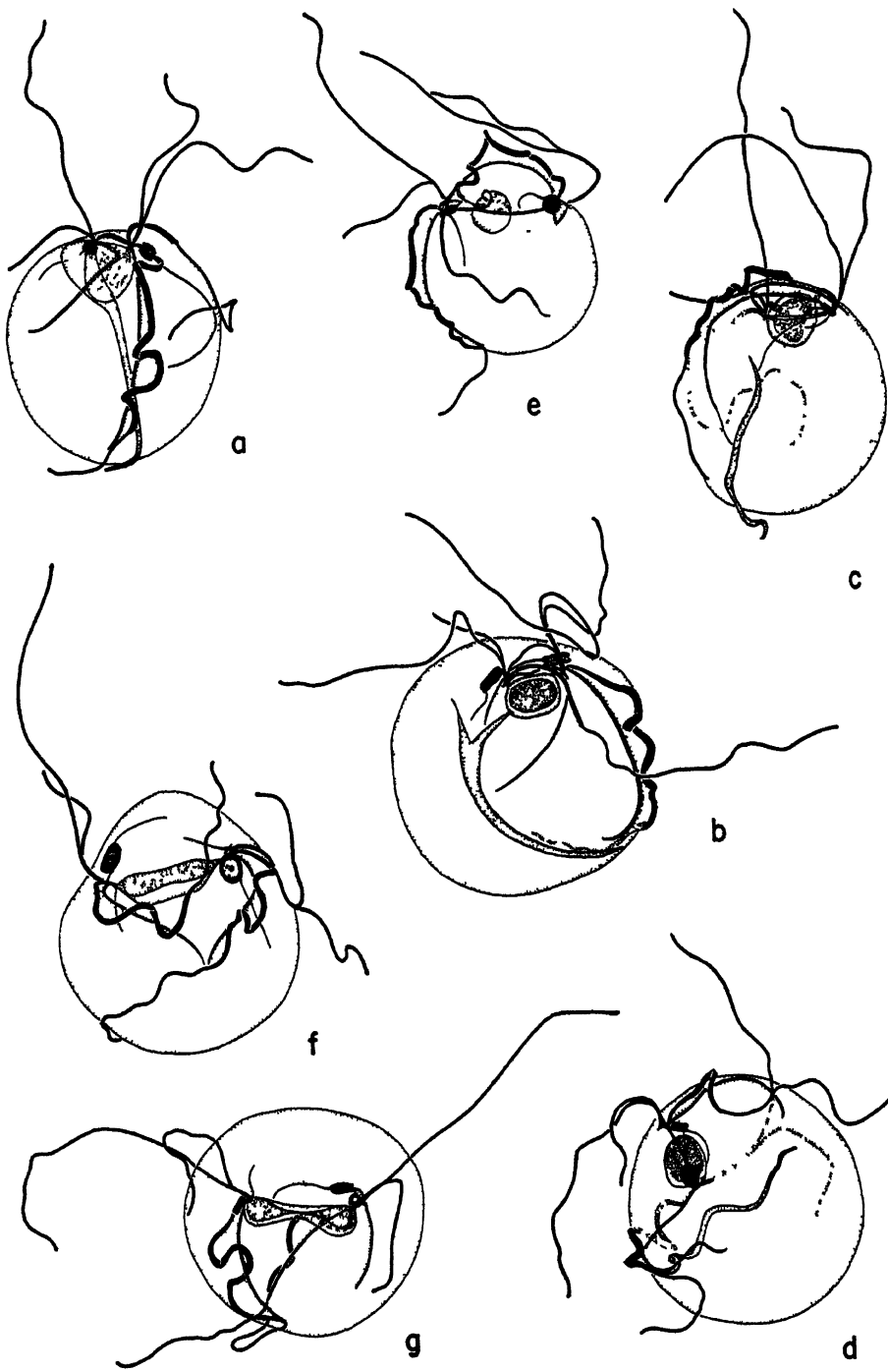


PLATE 17

Trichomonas prowazeki Alexeieff
from *Thamnophis elegans atratus*. Holl. Prot. $\times 2,650$
Division

a. Two daughter nuclei are present. Short stump of the third flagellum at each pole; new undulating membrane fully developed; daughter axostyles appear as lamellae; darkly impregnated paradesmose connects blepharoplasts. Actual distance between blepharoplasts, 8.75μ .

b. Two mastigonts at a 180° angle to each other; third flagella longer than those in a; new axostyle, at observer's left, shows enlarged capitulum and a tubular trunk; well-developed pelta applied to anterior part of capitulum.

c. Late division. Mastigonts almost fully developed; third flagella quite long; short stump of fourth flagellum at pole, to observer's right; axostyles did not reach their full length; posterior ends of axostyles not yet separated by membrane from surrounding cytoplasm.

d. Double individual with two sets of fully developed mastigont organelles; no paradesmose present. Note enlarged parabasal disk with two central granules and median constriction associated with one of the mastigont systems.

e. Triple individual with three fully developed mastigonts.

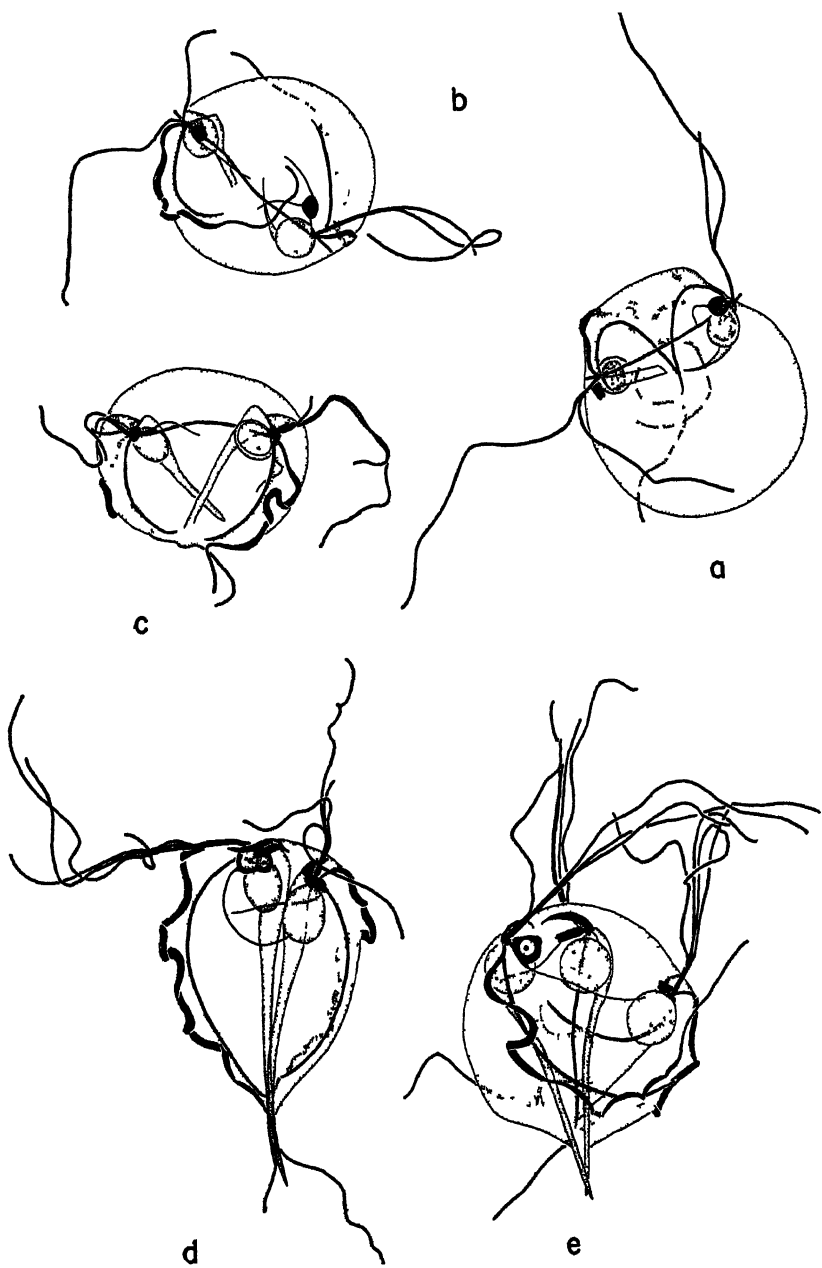


PLATE 18

Trichomonas brumpti Alexeieff
from *Testudo* sp. $\times 3,930$

a Composite diagram (right view), showing all mastigont organelles. Pelta rather inconspicuous in hematein stain; parabasal body represented by dorsally flattened disk, which fails to show central granule; no parabasal filament can be seen; nucleus contains numerous small granules and large spherical endosome surrounded by clear halo. A few bacteria may be seen in cytoplasm. Actual length of cytosome, 9.8μ . Holl. Hematein.

b. Composite diagram (right view), showing all mastigont structures. All organelles typical in appearance and arrangement. Note double free margin of undulating membrane, which, together with costa, follows a counterclockwise spiral course; discoid, dorsally flattened parabasal body with large central granule and long parabasal filament; spatulate capitulum of axostyle and crescent-shaped pelta. Holl. Prot.

c. Right view, showing all mastigont organelles. Holl. Prot.

d Apical view, showing relationship of mastigont organelles. Note particularly relationship of pelta to capitulum of axostyle. Large segment of parabasal body is covered by ribbon-shaped margin of undulating membrane. Holl. Prot.

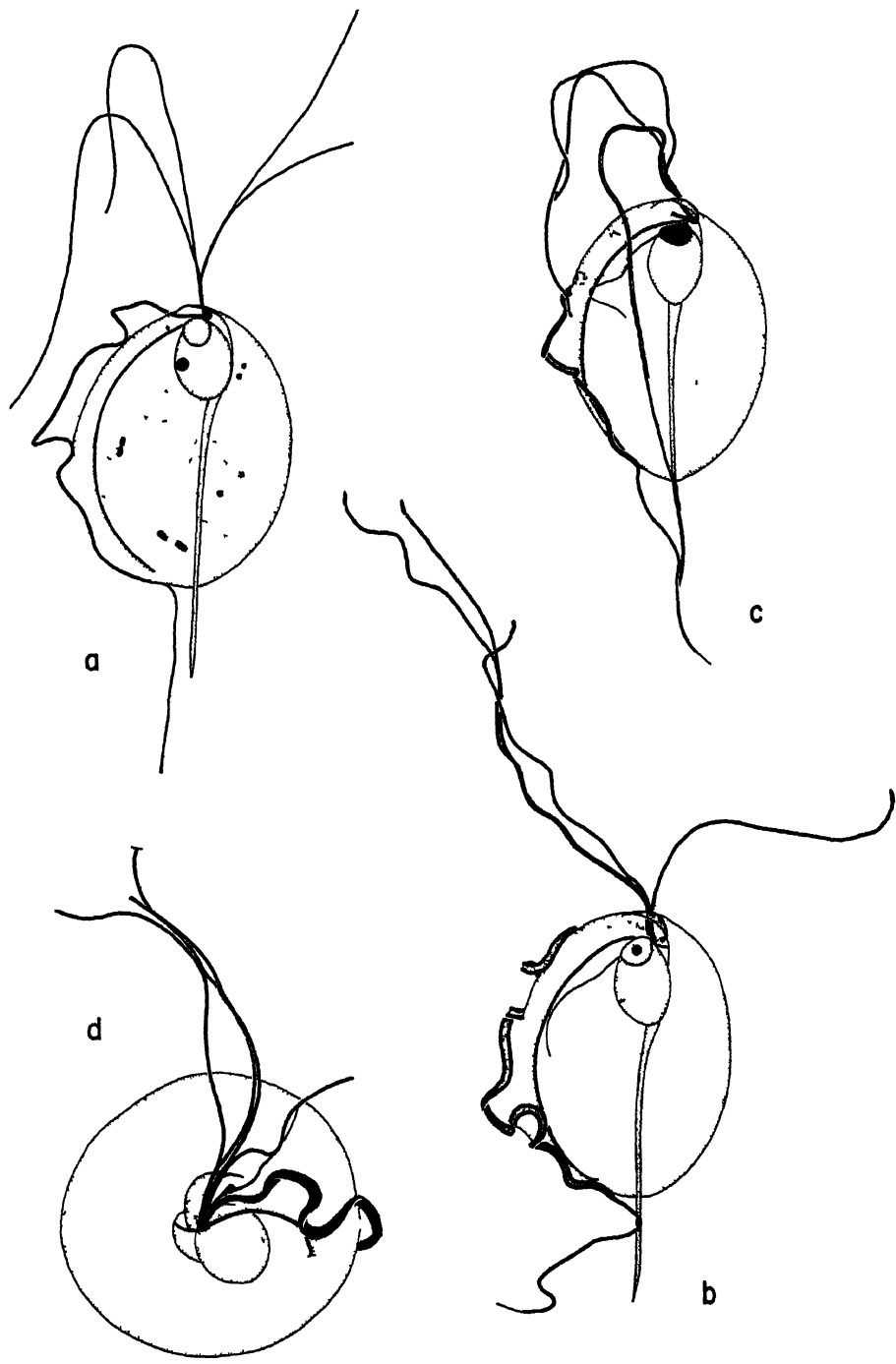


PLATE 19

Trichomonas brumpti Alexeieff
from *Testudo* sp. Holl Plot

a Left view, showing all mastigont organelles. Anterior flagella may be differentiated into two groups: the longer parental group (2 flagella) and the shorter daughter group (2 flagella). Parabasal body appears as a stout rod $\times 3,930$

b, c Anterior part of cytosome from left aspect, showing relationship of mastigont structures. In *b*, note relationship of pelta to capitulum of axostyle, filamentous dorsal extension of pelta, somewhat rhomboid shaped nucleus. Actual width of cytosome in *b*, $10\mu \times 3,930$

d, e Early division stages, showing two new parabasal bodies. In *d*, note two parabasal filaments associated with one of the disks. Actual width of cytosome in *e*, 7μ . *d*, $\times 3,930$, *e*, $\times 3,200$

f Ventral view of anterior part of cytosome of a young three flagellate, nondividing organism. Third flagellum still quite short $\times 3,930$

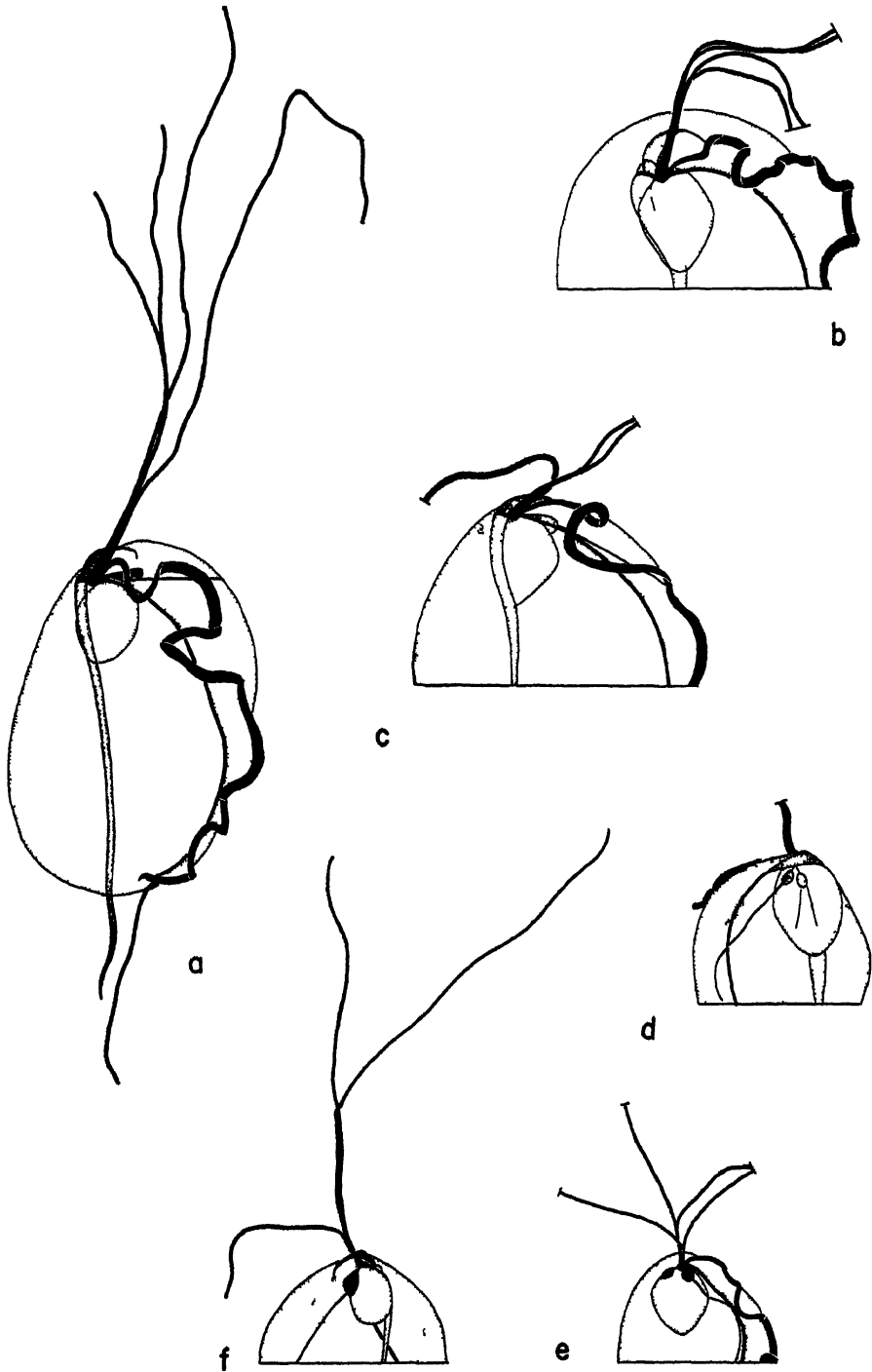


PLATE 20

Trichomonas brumpti Alexeieff
from *Testudo* sp. Holl. Prot.

a-n. Diagrams showing parabasal apparatus in different aspects. Depending upon intensity of impregnation and some intrinsic factors, the shape and internal structure of the parabasal disks may vary in appearance, even when viewed from a similar aspect (cf. *f-h*). The shape of the nucleus is also subject to variation. In *a* (ventral aspect) and *e* (dorsal aspect), proximal segments of anterior flagella, anterior part of axostyle, and pelta are shown. In *a*, part of costa and of undulating membrane are included. Parabasal disk shows two granules in *k*. Two filaments are associated with parabasal body in *m*. Actual length of nucleus in *c*, $3\mu \times 3,930$.

o. Early division. Daughter blepharoplasts some distance apart; two anterior flagella associated with each blepharoplast; new posterior flagellum already very long; new undulating membrane appears along proximal segment of the flagellum; large segment of parental axostyle still in cytosome. Actual distance between blepharoplasts, $2\mu \times 3,200$.

p. Nucleus probably in telophase. Still only two anterior flagella at each pole; discard of old axostyle no longer visible. $\times 2,650$.

q. Two daughter nuclei present. Still only two anterior flagella at each pole; new costa and new undulating membrane fully developed; new axostyles represented by lamellae. Actual distance between blepharoplasts, $11.5\mu \times 2,650$.

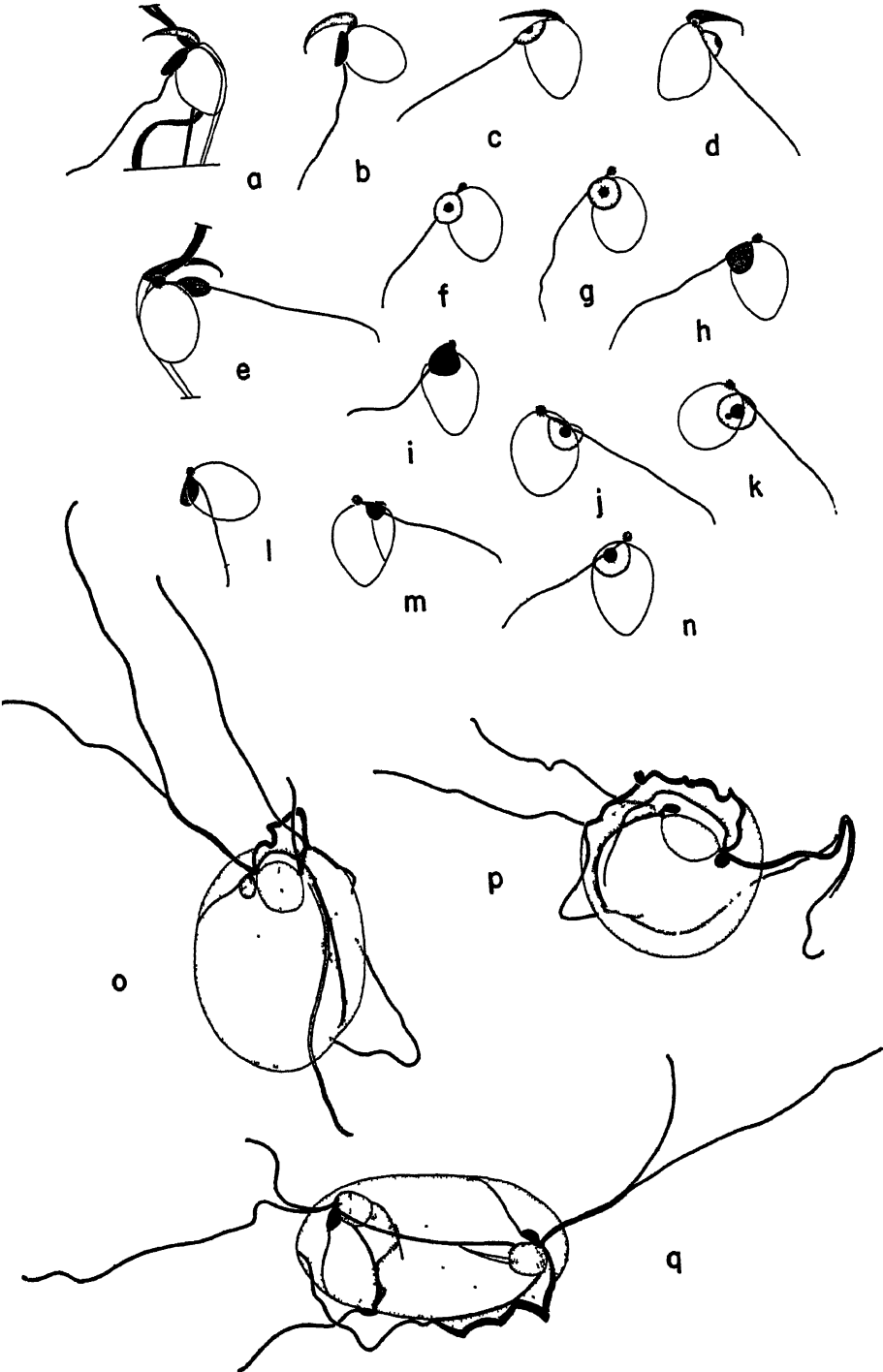


PLATE 21

Trichomonas brumpti Alexeieff
from *Testudo* sp. Holl. Prot.
Division

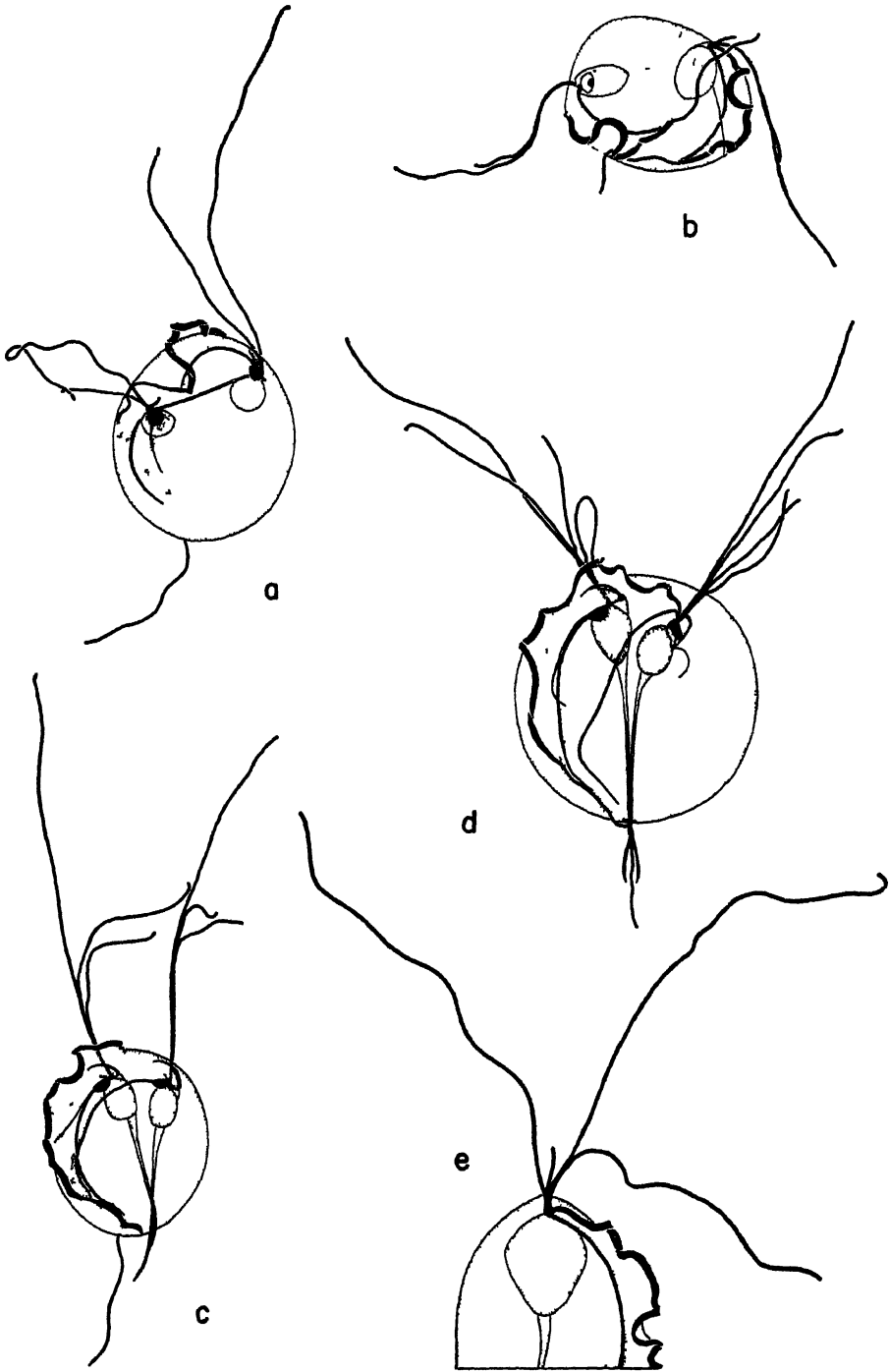
a. Somewhat later stage than in plate 20, *q*. Short stump of third flagellum at each pole. $\times 2,650$.

b. Very late division. Most mastigont structures fully developed; axostyles not yet at full length; rather conspicuous primordium of third flagellum at pole to observer's right. Actual distance between blepharoplasts, 7.5μ . $\times 2,650$.

c. A young double organism with two fully developed sets of mastigont organelles; only three anterior flagella present in each mastigont. $\times 2,650$.

d. A more mature double organism. Each of the two fully developed mastigont complements contains four anterior flagella. $\times 2,650$.

e. Anterior part of young, solitary, nondividing form. Fourth flagellum still much shorter than remaining three. Actual width of cytosome, 7.5μ . $\times 3,930$.



STUDIES ON PACIFIC COAST MOLLUSKS

I. ON THE STRUCTURE AND ADAPTATIONS OF *CRYPTOMYA CALIFORNICA* (CONRAD)

BY
C. M. YONGE

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ON THE STRUCTURE AND ADAPTATIONS OF *CRYPTOMYA CALIFORNICA* (CONRAD)

BY

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INTRODUCTION

VARIOUS CRAWLING lamellibranchs, species of the Leptonacea such as *Lepton squamosum* (Norman, 1891) and *Phlyctaenachlamys lysiosquillina* (Leyborne Popham, 1939), live as commensals, usually attached by a byssus thread to the walls, within burrows made by invertebrate inhabitants of muddy or sandy shores. But the only burrowing species known to be commensal in such burrows is *Cryptomya californica* (Conrad). As originally described by MacGinitie (1934, 1935), this animal, although living at depths of up to fifty centimeters, has extremely short siphons which open into the burrows of the crustaceans *Callinassa californiensis* and *Upogebia pugettensis* and of the cehiuroid *Urechis caupo*. Food and oxygen are obtained from the water which circulates through the burrow. *Cryptomya* is most usually associated with *Callinassa* where more food is available, the other two burrowers being themselves plankton feeders.

These observations of MacGinitie (which did not include any account of the structure of *C. californica*) were confirmed at the site of his own work at Elkhorn Slough, some thirty miles north of Pacific Grove, and elsewhere along the coast of central California. The genus *Cryptomya* is included in the family Myidae of the Myacea (Thiele, 1935) and hence closely related to the well known genus *Mya*, the species of which are characteristic deep burrowers with very long siphons, and to the allied *Platyodon cancellatus*, which bores into mudstone. *Cryptomya californica*, the only species of the genus, is widely distributed between 11° and 59° N latitude along the Pacific coast of America (Keen, 1937). The following brief account of this species appears justified in view of its unusual habits and the further light it throws upon adaptation within the Myacea.

This work was carried out at the Hopkins Marine Station, Pacific Grove, during the summer of 1949; it is a pleasure to acknowledge the help given by colleagues from the University of California, in particular Dr. R. I. Smith, Mr. D. P. Abbott and Mr. C. H. Hand. Subsequent technical assistance has been rendered at Glasgow by Dr. H. F. Steedman and Miss N. M. Davidson.

STRUCTURE

Shell.—The oval and almost smooth shell, which attains lengths of up to 3.0 cm., is shown in figure 1. It is obliquely truncated posteriorly with a slight gape. The valves are laterally compressed so that an animal 2.8 cm. long is only some 0.95 cm. thick. Internally the pallial line is well marked but there is no pallial sinus. The hinge is of the type characteristic of the Myacea, namely, with a large spoon-shaped tooth or chondrophore on the left valve (which is always slightly larger than the right valve) to which is attached the larger internal ligament or resilium.

Foot.—This organ is large and on occasion very active; as shown in figure 1, it

can extend up to three-quarters the length of the shell. The pedal gape through which it extends is correspondingly long (fig. 2); approximately one-third the length of the shell. The anterior and posterior pedal retractors, also shown in figure 2, are both well developed. When placed on a suitable substratum of muddy sand, the foot is protruded and the animal quickly burrows. Unlike *Aloidis* (*Corbula*) *gibba*, also a member of the Myacea and with very short siphons, no byssus thread is apparently ever produced in adult *Cryptomya*, although, as shown in figure 3, a groove and vestiges of a gland persist.

Siphons.—As stated by MacGinitie (1934, 1935), these are very short; indeed, his sketch (1935, fig. 11D, p. 685) gives an exaggerated idea of their length. Their

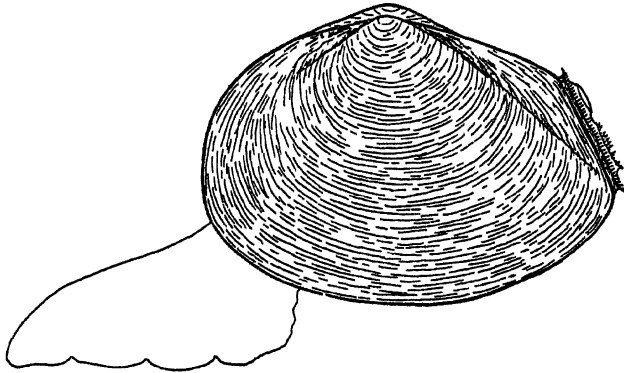


Fig. 1. *C. californica*, viewed laterally showing left side, with both foot and siphons fully extended. $\times 2\frac{1}{2}$.

appearance when fully extended is shown in figure 1. They protrude slightly upward from between the truncated ends of the shell valves. Owing to the lateral compression of the animal the siphons are oval in section, some five times higher than wide. They are also extremely short, the fused basal region being not longer than 1 mm. in the largest specimens. As in all the Myacea, the siphons incorporate the periostracal groove (Yonge, 1948) so that their surfaces, like all exposed regions of the mantle edge, are protected with periostracum. The middle lobe of the mantle edge is represented, as in all Myacea, by an outer ring of up to 80 simple outwardly directed tentacles, the inner lobe by a siphonal membrane around the exhalant aperture and by a row of not more than 30 simple, inwardly directed, but relatively widely spaced, tentacles around the inhalant opening. In life the animal lies buried with the outer ring of tentacles extending over the surface of the substratum, usually the wall of the burrow of *Callianassa*.

Ctenidia and feeding currents.—The structure of the ctenidia and the nature of their ciliary currents have not previously been described and both are of interest. The ctenidia are very large and almost fill the mantle cavity, as shown in figure 2. The inner demibranch reaches almost to the ventral surface of the mantle cavity while the outer demibranch is continued dorsal to the gill axis (fig. 2) forming a broad supra-axial extension (not shown in figure 3, which represents a section near the anterior end). The result of this disposition of the demibranchs is to provide an unusually extensive surface for the creation and filtering of water currents.

The gill itself is flat and homorhabdic and the ciliary currents created by the frontal cilia are exceptionally rapid. There is no food groove along the ventral margin of the outer demibranch but mucus-laden masses are carried toward the mouth along the gill axis and in the food groove on the ventral margin of the inner demibranch (figs. 2 and 3). On the surface of the demibranchs all material is conveyed to the ventral margins on both faces of the inner demibranch and on the outer face of the outer demibranch. But on the inner face of the latter, onto which (as shown in fig. 3) all material from its outer surface passes, larger particles are carried to the ventral margin (probably for eventual rejection) and smaller ones

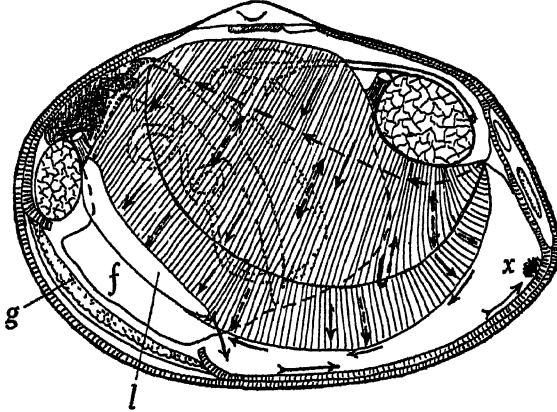


Fig. 2. *C. californica*, organs in mantle cavity viewed laterally after removal of left shell valve and mantle lobe. $\times 3\frac{1}{2}$. *f*, foot; *g*, glandular area bounding inner margin of pedal gape; *l*, labial palp; *x*, accumulation of pseudofaeces. Outline of gut and heart indicated by broken lines. Plain and broken arrows indicate direction of ciliary currents on exposed and under surfaces, respectively, of demibranchs; feathered arrows indicate direction of rejection currents from tip of palps and along midventral line where mantle edges fused.

to the axis (and so to the palps and mouth). Similar selective action by frontal cilia, due to the presence of different sizes of these beating in different directions, has been described by Atkins (1937) in a variety of lamellibranchs. In some it occurs on various surfaces, in others, e.g., *Barnea candida*, exclusively on the inner face of the outer demibranch as in *Cryptomya*. But *B. candida* differs from this species in the presence of an oralward current along the free margin of the outer demibranch. The arrangement of the food currents in *Cryptomya*, displayed semi-diagrammatically in figure 3, appears to be different from that of any species as yet described.

Labial palps and cleansing currents.—Apart from their length and narrowness the palps show no points of interest. Material rejected from their tips passes on to the surface of the mantle as do the larger particles or masses rejected from the gills, chiefly by way of the free margin of the outer demibranch. As shown in figures 2 and 4, all cilia on the lateral surfaces of the mantle carry particles ventralward. On either side of the pedal gape extend the elongated areas of mucous glands typical of the Myacea, e.g. *Mya* (Vlès, 1909), *Platyodon* (Kellogg, 1915) and *Aloidis* (Yonge, 1947). Cilia on the surfaces of these beat away from the pedal opening and so carry mucus into posteriorly directed currents along their outer

(or dorsal) margins. All particles from the anterior surfaces of the mantle are carried into these currents. Posterior to the pedal gape, along the midline ventrally where the mantle edges are fused, there is a powerful current into which are eventually drawn the mucus-laden masses which accumulate along each side and in

which these are conveyed posteriorly. All waste thus collects as masses of pseudofaeces (figs. 2 and 4, *r*) at the base of the inhalant siphon, through which these are periodically expelled.

Alimentary canal.—The course of this is shown in figure 2. A relatively long oesophagus communicates with a stomach into which projects an unusually large crystalline style. This lies within a separate style sac (i.e., like *Mya* and *Platyodon* but not *Aloidis*) the posterior end of which extends to the posterior end of the foot, where it joins the visceral mass. The style was observed to rotate (in anticlockwise direction when viewed from the anterior end) after the stomach was opened, but never more than nine times per minute, which is probably slower than in the intact stomach. In general anatomy the stomach resembles that of *Mya arenaria* (Yonge, 1923; Graham, 1949). There is a large dorsal pouch on the right side anteriorly and a large gastric shield covers the roof and right side of the stomach and bears conspicuous teeth anteriorly. The midgut is relatively short with only two loops, both near the beginning of its course (see fig. 2). The rectum traverses the ventricle before passing round the posterior adductor, the anus lying just within the exhalant aperture.

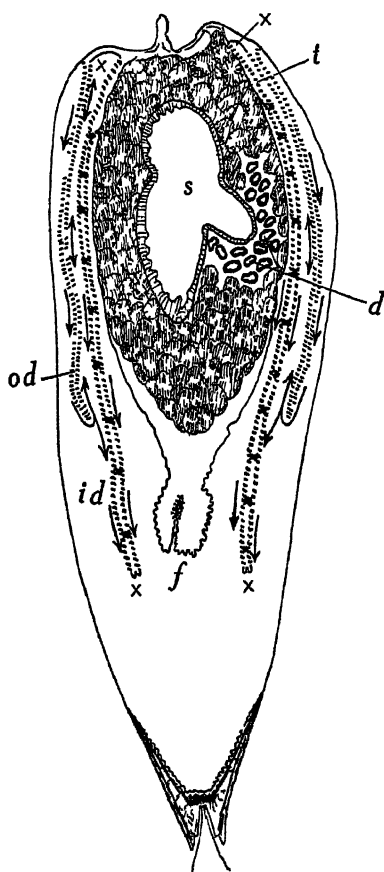


Fig. 3. *C. californica*, transverse section through center of visceral mass. $\times 50$. *d*, digestive diverticula; *f*, foot, showing groove and nonfunctional byssal gland; *id*, *od*, inner and outer demibranchs, former with marginal food groove; *s*, stomach; *t*, testis. Oralward currents indicated by crosses, currents on faces of demibranchs by arrows.

by *C. californica* may now be mentioned. For a member of the Myacea, this species is unusually active and has a very large foot and associated pedal gape and pedal muscles. In further correlation with activity, the shell is laterally compressed and has a superficial resemblance to that of members of the deposit-feeding Tellinidae and Semelidae, which are among the quickest of all lamellibranch burrowers (Yonge, 1949). The habit, possibly unique, of making contact with water for feeding and respiration by way of the burrows of *Callianassa* or, less often, of *Upogebia*

ADAPTATIONS

Although comparisons with conditions in other representatives of the Myacea will be deferred, the major adaptations possessed

and *Urechis*, represents a type of essentially deep burrowing which is not associated with the possession of long siphons and hence with possession of a pallial sinus. Here, therefore, examination of the shell alone gives no indication of the habits of the animal. The short siphons may be assumed to be primitive; it seems inherently improbable that long siphons once possessed would be lost. But whereas in deep-burrowing Myacea, such as species of *Mya*, long siphons are associated with a notable reduction in the size of the foot, the opposite is true in *Cryptomya*. Here the blood which is used to dilate the siphons in *Mya* is employed to extend the foot, essentially as in *Ensis* (Yonge, 1949).

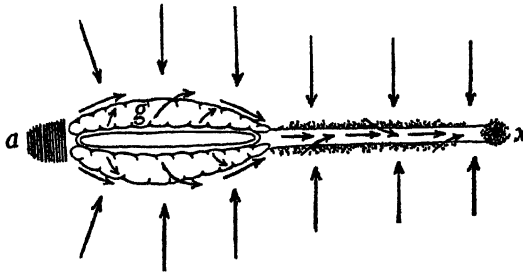


Fig. 4. *C. californica*, pedal gape with bounding glandular area (*g*) and fused ventral margins of mantle lobes viewed from above showing direction of rejection currents. *a*, anterior adductor; *x*, accumulation of pseudofaeces. $\times 3$.

The structure of both the inhalant siphon and the gills is modified in connection with the paucity of food (and lack of sediment) within the still water drawn in from the burrows, usually of *Callianassa*. The inhalant siphon is fringed with a single row of simple tentacles, resembling those of *Aloidis gibba* (Yonge, 1947), of little use for straining and so in contrast to the elaborate straining tentacles present in *Mya arenaria* (Morse, 1919) or in *Platyodon cancellatus*. The great size of the gills, increased by the supra-axial extension of the outer demibranchs, may reasonably be associated with the need for drawing in great quantities of water if an adequate amount of food is to be obtained. The presence of only two oralward food channels, along the gill axis and along the margin of the inner demibranch, the former carrying the finer particles, indicates that only limited supplies of food are obtained despite the very extensive straining surface. The cleansing mechanisms, although similar to those of *Mya* and *Platyodon* (Kellogg, 1915; Yonge, 1923) are simpler and devoid of the elaborate ciliary vortices present in these genera. The relative mobility of *C. californica*, rendered possible by the shape of the shell and the size of the foot, enables this species to move readily to new feeding areas. This may often be necessary owing to shortage of food but this disadvantage imposed by its mode of life is probably more than offset by the scarcity of enemies at the depths where it lives.

SUMMARY

Cryptomya californica, a member of the Myidae, occurs at depths of up to fifty centimeters but communicates by very short siphons with the cavities of burrows made by various large invertebrates, especially by *Callianassa californiensis*.

The shell is laterally very compressed and there is a large foot with powerful

pedal retractors and an extensive pedal gape. There is no byssus in the adult, which can burrow rapidly.

In correlation with the paucity of food and other suspended matter within the burrows, there is little straining action by tentacles around the inhalant opening and the gills expose very extensive surfaces for current production and the collection of food. There is no food groove on the free margin of the outer demibranch. Rejection mechanisms are simpler than in the allied species of *Mya* and *Platyodon*.

The alimentary canal is very similar to that of *Mya* with the same separate style sac. The style was observed to rotate.

C. californica is modified in various respects in connection with its unusual mode of life, which gives it protection from enemies but removes it from the major sources of food. Great ciliary activity while feeding and considerable mobility when new feeding areas have to be sought enable it to overcome this difficulty.

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STUDIES ON
PACIFIC COAST MOLLUSKS
II. STRUCTURE AND ADAPTATIONS
FOR ROCK BORING
IN PLATYODON CANCELLATUS (CONRAD)

BY
C. M. YONGE

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STRUCTURE AND ADAPTATIONS FOR ROCK BORING IN *PLATYODON CANCELLATUS* (CONRAD)

BY

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INTRODUCTION

THE CAPACITY possessed by various Lamellibranchia for boring into hard substrata would seem to have been preceded by specialization along one of a number of possible lines. One of these is certainly byssal attachment or "nestling," e.g., in Pilibranchia such as *Botula* and *Lithophaga* and in Eulamellibranchia such as *Hiatella* (*Saxicava*); another is deep burrowing into soft substrata. An excellent example of the latter is provided by *Platyodon cancellatus*. This, the sole species of the genus, is included in the family Myidae of the Myacea (Thiele, 1935) being closely allied to *Mya*, a genus with which it has been, and still sometimes is, associated. But the differences do appear great enough to justify the erection of a separate genus although *P. cancellatus* can justifiably be regarded as a *Mya* which has become adapted for boring into soft rock.

The observations here recorded were made at the Department of Zoölogy, University of California, Berkeley, during 1949 when the author was Visiting Professor. He has particular pleasure in recording his indebtedness to Professor H. Kirby, Chairman of the Department, and his other colleagues, for their help and friendship. Especial thanks are due to Dr. R. Stohler for guidance in collecting.

HABITAT AND STRUCTURE

Platyodon cancellatus occurs only along the shores of California, between 33° and 38° N latitude (Keen, 1937), where it is found at about midtide levels, but exclusively where there are areas of relatively soft mudstone. It bores into rocks of this type and may be extremely numerous within them. The main sources of supply were Duxbury Reef and Frenchman's Reef, both on the open coast and some miles respectively north and south of San Francisco.

Shell.—No attempt was made to discover very small specimens which probably settle within previous borings or in other depressions, but individuals obtained by breaking open rocks varied in shell length between about 3.0 and 6.5 cm. The shell is stout with concentric ridges (except where these are worn away as described below). As shown in figure 1, the shell is roughly oval and is truncated posteriorly where there is a wide gape into which the siphons can be withdrawn when the shell closes (fig. 2,B). Viewed laterally, the shell resembles that of *Mya truncata* but is more rounded in cross section (figs. 2,A and B). The hinge is situated about one-third of the length of the shell from the posterior end* (unlike *Mya arenaria* where it is almost centrally placed). It possesses internally the large chondrophore attached to the left valve which is characteristic of the

* There is some evidence that the posterior regions of the shell valves become proportionately somewhat greater in larger individuals. This might be due to greater ease of growth posteriorly where the shell is not firmly wedged in the boring.

Myacea. But this structure and the internal ligament or resilium associated with it are reduced in comparison with those in the burrowing *M. arenaria*. Moreover, in the latter (and in *M. truncata*) the left umbo is somewhat lower than the right one, under which the chondrophore extends. In *Platyodon*, however, the two are the same height, partly owing to the reduction of the chondrophore but mainly to the forcing of the right umbo within the base of the left umbo (shown in fig. 2), presumably a result of pressure within the boring.

Siphons.—These are stout (fig. 1) and can extend to lengths somewhat greater than the shell. As in all Myacea, they are formed from the inner and middle lobes of the mantle edge together with the periostracal groove. Hence they are covered with a strong periostracum although this is smoother than in *Mya arenaria*; in *M. truncata* it is exceptionally thick and wrinkled. But the periostracum in *Platyodon* is locally thickened to form four scalelike areas (fig. 1) arranged two on each

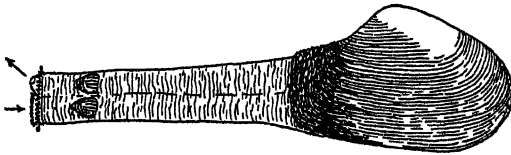


Fig. 1. *P. cancellatus*, viewed from right side after removal from boring, with siphons fully extended. Latter covered with periostracum forming "scales" near posterior end. Shell ridged and covered with thick periostracum posteriorly but worn smooth around umbones. $\times \frac{1}{2}$.

side and situated some little distance from the tip of the siphons. Each consists of a thickened pad with a pointed, flexible, and corrugated tip. In an animal of shell length 6.0 cm., the siphons extended for 7.0 cm. and these "scales," which were 0.9 cm. long, were 1.0 cm. from the tip. When the siphons are withdrawn their extremities are intucked up to the region of the scales. The distal, flexible regions of these also bend inward so that the fully contracted siphons when viewed from behind (fig. 2,B) terminate in a characteristic cross grooving.

The openings of the siphons resemble those of other Myacea, i.e., there is a common outer ring of tentacles, a siphonal membrane around the exhalant opening and further rows of tentacles around the inhalant aperture. The outer tentacles consist of an inner row of large with outer and intermediate rows of smaller tentacles. All extend outward when expanded. The tentacles around the inhalant opening are of particular interest. They are in six rows, the largest being deepest within the margin, and are arranged in alternating rows like cycles of septa. The two largest (i.e., innermost) rows consist of 12 tentacles, the third of 24, the fourth of 48, while the very numerous small tentacles of the last two rows are difficult to count or to allocate. The tentacles of the four innermost cycles are all distally pinnate. They bend inward with the twelve innermost and largest meeting in the centre and the tentacles of succeeding cycles interdigitating in series outwards. The incurved tentacles thus form a highly complex basketwork which acts as a filter guarding the entrance into the mantle cavity. This filter is much less complex in species of *Mya*, but these live in relatively quiet waters whereas *P. cancellatus* occurs on exposed coasts where there is much surf and consequent turbidity. The membrane surrounding the exhalant opening is tubular, is directed somewhat dorsally, and is very sensitive.

Mantle cavity and ciliary currents.—The organs within the mantle cavity, which have already been described and figured by Kellogg (1915), very closely resemble those of *Mya arenaria*. The anterior adductor has moved somewhat ventrally in conformity with the dilation of the anterior half of the shell and this has caused a corresponding displacement from the anterior to the ventral surface of the foot and the pedal gape. Both are very small, appreciably less than in any species of *Mya*, the gape being 0.8 cm. long in an animal of shell length 5.9 cm. But despite its small size the foot was observed occasionally to protrude through the pedal gape. It could then clear particles from the unciliated lips of the gape but no other function can be ascribed to it. In adult life it certainly plays no part either in locomotion or in boring.

The gills and palps are very similar to those of *Mya arenaria* (Vlès, 1909; Kellogg, 1915; Yonge, 1923) and the ciliary currents upon them follow the same course. On the mantle surface all particles are carried into vortices, one on either side of the pedal gape, which, as in other Myacea, is bordered internally by elongated areas of mucous glands (Kellogg, 1915). Waste matter is finally conveyed posteriorly along the midline ventrally where the mantle edges are fused. Pseudofaeces collect as usual at the base of the inhalant siphon prior to ejection.

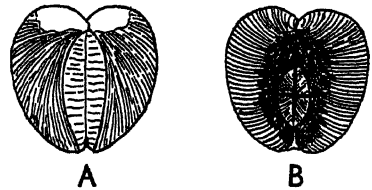


Fig. 2. *P. cancellatus*, viewed from both ends when contracted. $\times 1$. A, anterior end showing gape, exposed mantle edge being covered with periostracum, abrasion of shell round umbones and manner in which right umbo fits into left; B, posterior end showing appearance of siphonal "scales" when in-tucked, also presence of periostracum on shell valves where no abrasion.

BORING

Nature of borings into rock.—Kellogg (1915) refers to *Platyodon* as a nestler found in burrows of soft sandstone. But the very numerous specimens examined around San Francisco were all true borers and found always in mudstone. This is friable when thoroughly dry but *in situ* can only be broken after hard and repeated blows with a geological hammer. When the borings were opened the animals were always found fitting exactly and very tightly within. Although the young must certainly settle in depressions or in old borings in the rock, there is clear evidence that as the animal grows it excavates its own boring and that it is modified in connection with this mode of life.

The form of the borings can best be described by reference to figure 3. The borings conform to the shape of the shell with extended siphons, the latter reaching to the opening but never beyond. The deep portion of the boring, where the shell is situated, has pronounced ridges corresponding to the dorsal and ventral sides of the shell. These are shown in section in figure 3, B. The "dorsal" ridge is the more prominent, especially in the region of the umbones, where the shell cuts deeply into the rock. The surface of the boring here is fairly smooth although there are many small irregularities due to the varying texture of the rock. The area at the base of the siphonal region is rougher and there are accumulations here of mud and fine fragments of stone. The walls of the siphonal extension are always extremely smooth. When borings were carefully opened to expose limited areas

of the animals *in situ*, these were always found tightly wedged inside. The siphons were withdrawn but the shell valves were separated ventrally for distances of up to 0.5 cm. owing to dilation with blood of the thickened and fused mantle edges. As a result the shell valves were pressed firmly against the walls of the boring.

Method of boring.—The form of the boring provides clear evidence that it has been excavated mechanically by the shell valves. These bear many fine ridges,

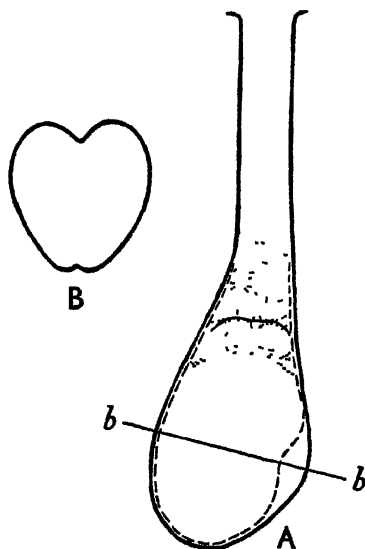


Fig. 3. *P. cancellatus*, form of boring. $\times \frac{1}{2}$. A, boring opened longitudinally, broken line indicating extent and depth of ridge, stippled area region around posterior end of shell and base of siphons where mud and fine particles accumulate, position of posterior end of shell valves shown by curved line; B, section through boring along line marked *b-b* in A, showing extent of "dorsal" and "ventral" ridges (cf. fig. 2).

more numerous and rougher than those of species of *Mya*. The ridges are worn smooth in the regions below and in front of the umbones (see fig. 1), where deep grooves are cut on either side of the "dorsal" ridge in the burrow (fig. 3). The very thick periostracum, moreover, is completely worn away except at the posterior end of the shell (figs. 1 and 2,B), where alone the shell does not bear against the rock, from which it is separated by the accumulations of abraded particles already mentioned and indicated in figure 3,A. There is little accumulation of mud in other areas.

In the process of boring, the shell valves must rock on the fulcrum of the hinge, which consists of the single tooth (chondrophore) with its localized internal ligament. This rocking motion was observed to occur after animals had been exposed in their borings. Moreover, when the siphons are withdrawn the anterior adductor contracts, causing the posterior halves of the shell to separate and so widen the gape for the reception of the siphons. The posterior adductor may similarly contract, causing divarication of the anterior halves of the shell. In

an animal of shell length 5.3 cm., the anterior separation of the valves so caused was 0.8 cm. The results of these contractions are shown in figure 2,A and B. The ventral separation of the shell valves, due to dilation with blood of the enlarged and fused mantle edges, must further aid in the gradual abrasion of the rock surface by the shell.

Similar movements are executed in the course of burrowing into stiff mud by *Mya arenaria* or into even stiffer clay by *M. truncata*. But they are more pronounced in *Platyodon* owing to the reduction in size of the chondrophore and ligament and also to their posterior migration which results in the contraction of the posterior adductor causing correspondingly greater separation of the anterior halves of the shell valves. These are the major agents of boring as revealed by the effects of the abrasion upon them. There is apparently no passage of rock fragments into the mantle cavity as there is in the much more specialized Pholadidae—and also in the less specialized *Hiatella* (*Saxicava*), as shown by Hunter (1949). Instead these fragments are moved back until they accumulate around the posterior region

of the shell, where it does not fit tightly because of continued forward boring. The function of the small pedal gape is dubious (the very reduced foot having no apparent function other than to keep the gape clean) but it will permit the passage of some water from the mantle cavity when the siphons are suddenly withdrawn.

The extremely smooth surface of the siphonal extension of the boring is due to the four periostracal scales on the siphons, which rub against the boring when the siphons extend or contract. Their presence is obviously correlated with habit. The only comparable structures in the Lamellibranchia are the pair of periostracal thickenings, one on each side of the tip of the siphons, in *Mya truncata* and *Schizothorus nuttalli*. The former belongs to the Myidea, the latter to the Mactridae, but both are deep burrowers with long siphons incorporating the periostracal groove. The local thickenings of periostracum protect the ends of the siphons and may assist in pushing a way through the substratum if the opening of the burrow becomes obliterated. In short, local thickenings of periostracum may be formed on the siphons and have survival value when the animal lives deep within a stiff or hard substratum.

Evolution of the boring habit.—*Platyodon* is essentially a modified deep burrower, very closely allied to *Mya*. Although *M. arenaria* has been introduced along the Pacific coast the only indigenous species of this genus is *M. truncata*, which extends from the far north down to Puget Sound, actually from 72° to 48° N latitude (Keen, 1937). This species, at any rate in the Atlantic, occurs frequently in heavy clay and is certainly adapted for burrowing into much stiffer substrata than is *M. arenaria*. *P. cancellatus* represents a further stage in the same line of adaptation. Since it occurs exclusively in the Pacific, it may well have originated from the same stock as *M. truncata*.

The highly specialized Pholadidae and Teredinidae grip the end of the boring with the suckerlike foot, which has been displaced to the anterior end, and then rotate, first in one direction and then in the other, as they bore their way into rock or stone, e.g., *Zirfaea* (MacGinitie, 1935), *Teredo* (Miller, 1924). In consequence the boring is circular in section. *Hiatella* (*Saxicava*), although much less modified, also excavates a circular boring. The slender foot may be protruded around one side of the shell and a single byssus thread be attached to the wall of the boring (Hunter, 1949). Later contractions of the pedal muscles cause the animal to twist round in the burrow. But *Platyodon* has no such freedom within the boring and resembles in this respect the otherwise very different *Tridacna crocea* (Yonge, 1936) and also species of *Botula* (to be described in a later paper). Like these animals, *Platyodon* does not rotate, all species working their way straight into the rock. But whereas they are attached by a byssus throughout life *Platyodon* is not. It would seem, indeed, to bore into the rock in the same manner in which *Mya arenaria* and *M. truncata* burrow, i.e., going straight in, as evidenced by the presence of the "dorsal" and "ventral" ridges in the borings. Similar ridges have been observed by the author in the burrows made in stiff clay by *M. truncata*.

This explanation is, however, incomplete. In both species of *Mya* the foot, though much reduced in the adult, remains capable of gripping the substratum when the animal burrows. It provides the necessary purchase—at any rate when they are moving through suitable substrata, because it is very doubtful whether *M. truncata*

can move in this way through the dense, stiff clay into which it certainly penetrates. The problem is to explain how *Platyodon* obtains the purchase necessary for boring. This is done by means of the byssus in *Tridacna crocea*, *Botula* spp. and to some extent in *Lithophaga* spp., by aid of the suckerlike foot in the Pholadidae and the Gastrochaenidae, and in *Hiatella* by distension of the basal regions of the siphon (Hunter, 1949). But in adult *Platyodon* there is neither byssus nor attaching foot and the shape of the boring does not permit the siphon to be distended basally as in *Hiatella*.

Unfortunately nothing is known of the life history of *Platyodon* but it is reasonable to assume that after settlement it has a byssus, as *M. arenaria* has at this stage (Kellogg, 1899) and as the allied *Aloidis gibba* and *Sphenia binghami* have throughout life (Yonge, 1947, 1951). Initial byssal attachment within an old boring or other depression on the rock (it would be interesting to know by what means, if any, mudstone is detected) could then be followed by boring, for which the form and sculpture of the shell and the posterior migration of the hinge both fit the animal. Once the boring was formed to the extent that the shell was trapped within it, being wider than the siphonal extension, then the byssus would become unnecessary. Owing to the posterior migration of the fulcrum of the hinge, contraction of the posterior adductor causes wide separation of the anterior halves of the shell (see fig. 2,A) so that these become the major agents of boring. But they need to be pressed firmly against the rock. There is no evidence that this pressure is provided by the siphons, as it is in *Hiatella*, although the scales on these will certainly serve to enlarge the siphonal extension as growth proceeds. Pressure more probably comes from distension of the fused and greatly enlarged ventral mantle edges. When distended with blood they certainly force the shell valves apart and must also tend to push them upward (morphologically) which is indicated by the deep grooves cut by the umbones on either side of the prominent "dorsal" ridge. Some such mechanism probably accounts for the penetration of *M. truncata* through stiff clay and is a prime factor in forcing the shell valves apart in deep burrowers where the unaided ligament would fail to counteract the lateral pressure of the substratum. It is noteworthy that *Panope generosa*, the deepest of all deep burrowers, has the thickest ventral mantle edge.

Platyodon may therefore be assumed to have taken to boring, albeit in soft rocks, after adaptation as a deep burrower in stiff substrata like those which may be penetrated by *M. truncata*. Judging from the occurrence of *Zirfaea gabbi* in hard blue clay, in which its slow rotating movements have been fully described by MacGinitie (1935), the Pholadidae probably also originated as burrowers in stiff substrata before exploiting, most successfully, the possibilities of boring into all but the hardest rocks. But they were preadapted to a greater extent than *Platyodon*, which is confined to the softest rocks.

SUMMARY

Platyodon cancellatus, a member of the Myacea, occurs intertidally along the coast of California but always where there are areas of mudstone, into which it bores.

Borings are excavated mechanically by the shell, which is ridged, and in which, compared with *Mya*, the hinge is displaced posteriorly and the chondrophore reduced. Four scalelike thickenings of the periostracum which covers the siphons are responsible for increasing with growth the size of the siphonal extension to the boring.

The organs in the mantle cavity and the ciliary currents concerned with feeding and with cleansing are all similar to those in species of *Mya*. The tentacles around the inhalant aperture are more numerous and complex and form an extremely efficient filter. This is probably correlated with the high turbidity in the exposed habitat.

P. cancellatus bores straight into the substratum without twisting; the boring is therefore not round in section as it is in the Pholadidae. There are "dorsal" and "ventral" ridges within the boring, the former being very pronounced between the umbones of the shell. The mode of boring is essentially similar to that of burrowing in stiff substrata by species of *Mya*. The necessary pressure against the rock is probably obtained initially by byssal attachment and later by distension with blood of the thickened and fused ventral mantle margins.

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STUDIES ON
PACIFIC COAST MOLLUSKS

III. OBSERVATIONS ON
HINNITES MULTIRUGOSUS (GALE)

BY
C. M. YONGE

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OBSERVATIONS ON HINNITES MULTIRUGOSUS (GALE)

BY

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University of Glasgow

INTRODUCTION

THE SPECIES *Hinnites multirugosus* (Gale) (= *H. giganteus* Gray) is probably not uncommon intertidally along the Pacific coast of North America. But its habitat, usually under ledges and in rock crevices, often makes it hard to find. Smith and Gordon (1948) record it as occurring from low-tide level to depths of 12 fathoms at Monterey while Keen (1937) states that it ranges horizontally between 25° and 54° N latitude. It is of particular interest because after initial free existence it becomes cemented, unusually late in development, to the substratum. For this reason comparisons are of interest on the one hand with related species that remain free throughout life, such as *Pecten maximus* or *P. tenuicostatus*, and on the other with species of genera which become cemented at earlier stages in the life history, e.g., *Spondylus* and *Plicatula*. These observations were recorded at the Hopkins Marine Station, Pacific Grove, during the summer of 1949. Thanks are due to Mr. D. P. Abbott and Mr. C. H. Hand for collecting many of the specimens examined.

YOUNG (UNATTACHED) STAGES

Several small free individuals were found in rock pools. The largest of these measured 1.1 cm. along the hinge line, 1.9 cm. from the umbo to the free margin of the shell (conveniently rather than accurately to be referred to as the dorso-ventral diameter) and was 1.5 cm. long (i.e., anteroposterior diameter). These measurements indicate the typical proportions, the dorsoventral diameter being the greater. This also applies to species which retain byssal attachment throughout life, e.g., *Chlamys varia*; only in those that lose all attachment, such as the species of *Pecten* mentioned above, is the anteroposterior diameter the greater. In this young *Hinnites* the maximum distance between the internal surfaces of the valves was 0.6 cm. As shown in figure 1, the auricles are asymmetrical, the portion anterior to the umbo being the longer. At this stage the animals are largely bilaterally equivalvular. The two valves have approximately the same curvature, but the right valve, on which the animal normally rests, is colourless and spinous only on the auricles, whereas the left one is medium brown with small spines along the course of the ridges that radiate from the umbo. There is a deep byssal notch on the anterior auricle of the right valve (not shown in figure 1) and this allows passage for the foot (*f*) and the byssus. This valve, at any rate in the later stages of free life and possibly in connection with the approach of attachment, is very slightly wider than the upper one. The anterior auricle on the right valve is also somewhat the larger, the hinge line, as shown in figure 1, being displaced onto what is functionally the upper side of the shell.

In life the shell valves gape widely; to a maximum of 1 cm. in the specimen here described. As shown in figure 2, the inner mantle lobes or pallial curtains (*p*)

are unusually deep; they are also very mobile, thus controlling, as in allied genera, the extent and site of the inhalant and exhalant openings. They carry a fringe of short tentacles, and the middle mantle lobes, which normally protrude a little beyond the margin of the shell, bear both eyes and tentacles. Some of the latter were observed to extend for distances of 1.2 cm. beyond the margin of the shell (see fig. 1).

In habits the young *Hinnites* resembles allied species of *Pecten* or *Chlamys*. If an animal is placed on its left valve the foot is immediately extended and bent under the shell, holding on to the substratum although without forming a byssus.

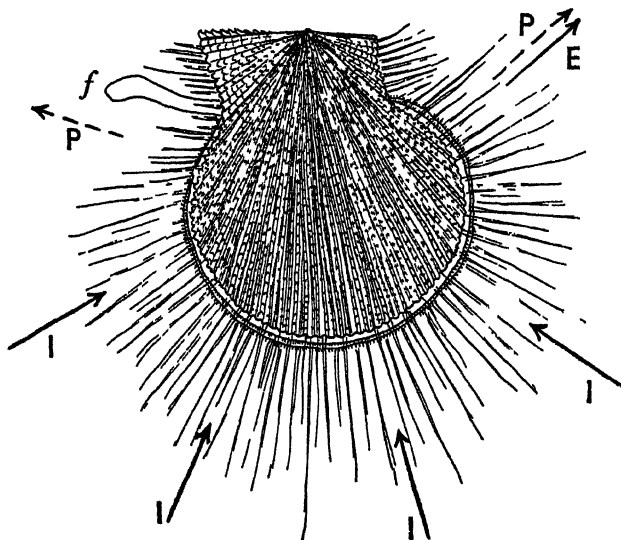


Fig. 1. *Hinnites multirugosus*, young unattached specimen viewed from above, i.e., with left valve uppermost. $\times 2\frac{1}{2}$. *f*, foot; *E*, *I*, arrows indicating exhalant and inhalant currents; *P*, broken arrows indicating regions where pseudofaeces rejected from mantle cavity.

The mantle edges and tentacles are then withdrawn and a sudden contraction of the adductor causes extrusion of water along the free margin of the shell. Were the shell free it would, as a result, move backward, i.e., execute the escape movement with hinge foremost, but because the foot is attached the effect of this extrusion is to cause the shell to turn over along the hinge line. This reaction was often seen and always occurred between 15 and 60 seconds after the animal had been turned over. Swimming movements, i.e., with the free margin of the shell valves in front, were also performed, precisely as in species of *Pecten* (Yonge, 1936). But young *Hinnites* are usually attached by a byssus. The foot is extruded, as indicated in figure 1, and attachment is quickly made by means of a transparent byssus consisting of a central mass ending in a number of fine threads. This appears to be the normal habit in life, swimming or escape movements taking place only if the animal is dislodged by the very powerful water movements to which it may be subjected.

When attached, the shell valves gape widely and an inhalant current (fig. 1, *I*) is drawn in over a wide area from the base of the anterior auricles around the free

margin of the shell to a point about midway along the posterior surface. Here, as shown in figure 2, the tips of the ctenidia (*c*) make contact with the inner mantle lobes, thus establishing a functional separation between inhalant (I) and exhalant (E) openings. The latter is restricted and the current flows through it in a "posterodorsal" direction. Both faeces (see position of anus (*a*) in fig. 2) and waste from the mantle cavity are carried out in this current, but larger collections of waste, or pseudofaeces, accumulate anteriorly where they are periodically ejected following contractions of the adductor muscle. As recorded elsewhere (Yonge, 1936) for *Pecten*, it is the direction of these ejections of water, on either side of the hinge line (fig. 1,P), which makes swimming movements possible. The ctenidia can easily be observed between the widely opened shell valves. They are extremely mobile, extending and withdrawing as a result of muscular action, while particles were seen to be carried very rapidly oralward along the axes.

ATTACHMENT

Attached individuals are usually irregular in shape because the shell conforms to the irregularities of the surface of the rock. The unattached (left) valve becomes worn and is usually covered with encrusting growths so that it is impossible to determine the area of the shell prior to attachment. But the right valve is protected from wear and overgrowth and so the size at which attachment occurred can be measured. In the specimens examined the dorsoventral diameter of the shell at the time of attachment varied widely—from 2.2 to 4.2 cm.

What stimulus causes attachment is unknown; it seems likely to be intrinsic and might well be connected with the onset of sexual maturity. The process would seem to consist of an extension of the right mantle edge so that the secretion of new shell substance by the outer (secretory) lobe flows onto and fuses with the substratum. Sections of the mantle edge of both attached and free valves failed to reveal any structural differences between them. The form of the attached valve inevitably conforms with that of the substratum, although, especially in larger specimens, eventual additions to the shell may be free—should, for instance, the shell grow out over the edge of a ledge of rock. The shape of the free valve tends to conform in its turn with that of the attached one, so that both the outline and the surface of the larger specimens of *Hinnites*, which may exceed 14 cm. in dorsoventral diameter, are often most irregular. If anything, the dorsoventral diameter tends to become relatively greater; the attached valve becomes more concave, in most adults being twice as deep as the left valve.

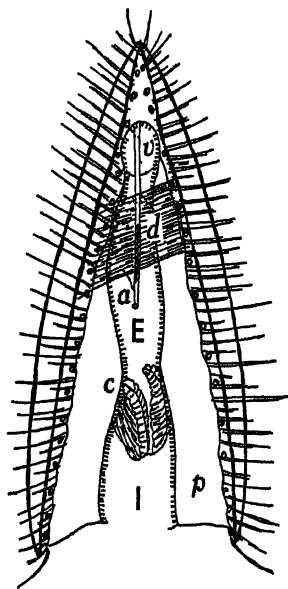


Fig. 2. *H. multirugosus*, young specimen viewed from posterior end. $\times 4$. E, I, exhalant and inhalant chambers; *a*, anus; *c*, posterior tips of ctenidia, making functional contact with palial curtain (*p*); *d*, adductor; *v*, ventricle (stippled).

ADULT STRUCTURE

The appearance of the adult animal lying within the attached valve is shown in figure 3. There is a close resemblance to other species of *Pecten*, of which, following Thiele (1935), *Hinnites* is here considered to be a subgenus. Apart from the figure, therefore, description can largely be confined to comparison with *Pecten tenuicostatus* (Drew, 1906), *P. maximus* (Dakin, 1909) and *P. irradians* (Gutsell, 1931).

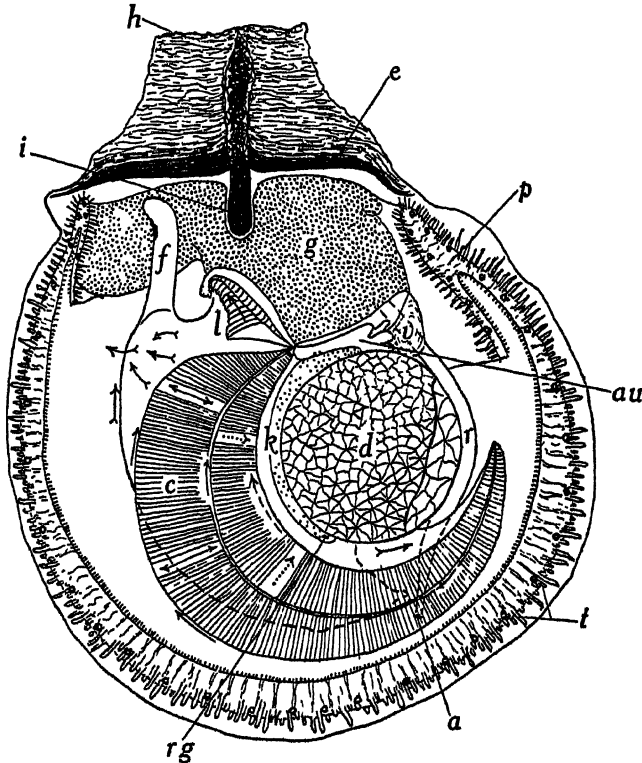


Fig. 3. *H. multirugosus*, adult viewed from above after removal of left shell valve and mantle lobe. $\times 1$. a, anus; au, auricle; c, left ctenidium; d, adductor; e, external ligament; f, foot; g, gonad (stippled); h, initial hinge line; i, kidney (stippled); l, labial palps; p, pallial curtain in region of posterior fusion; r, rectum; rg, reno-genital pore; t, tentacles of middle, sensory lobe of mantle edge, eyes also shown; v, ventricle. Feathered arrows indicate rejection currents, other arrows currents on ctenidia and palps; broken arrow current on ctenidial axis, dotted arrows current produced by abfrontal cilia on descending filaments.

H. multirugosus has separate sexes, the white male gonad contrasting with the red ovary. Both gonads extend into the mantle anterodorsally (fig. 3, g) and open to the exterior by way of a common renogenital pore (rg). The kidneys communicate with the pericardium on the dorsal* side of the large adductor muscle (d), and with the exterior on its ventral side. This muscle is composed for the most

* Although adopted throughout this paper, the use of dorsal and ventral is not strictly correct; although the umbo is often taken as a fixed point and regarded as being always dorsal, there are valid reasons for considering the foot as a fixed ventral point, the other tissues (including the umbo) moving in relation to this point.

part of striated, i.e., "quick," muscle fibres. The ctenidia are attached by way of a suspensory membrane and resemble in this and all other respects those of allied species described by the above authors and also by Ridewood (1903) and Atkins (1937). They possess abfrontal cilia. The labial palps and frilled lips are also similar. The alimentary canal follows the same course but the anus (*a*) lies at the end of a longer papilla which projects from some distance ventral to the adductor as shown in figures 3 and 4. In *Pecten maximus*, on the other hand, the papilla is short and the anus is on the lower side of the posterior surface of the adductor; in *P. tenuicostatus* it is curled upward so that the anal opening lies well to the posterior (Drew, 1906; Kellogg, 1915). The foot is reduced but not significantly more than in the motile species of *Pecten* and *Chlamys*. The mantle edge is elaborate as it was in the young individuals and is in all related species. The pallial curtains are very deep, up to 1.5 cm. in a specimen of approximately 8 cm. shell diameter. The curtain is fringed with short tentacles and is deep-orange colored, the appearance, when viewed between the separated valves, being very handsome. There is a concentration of black pigment on the inner side of the curtains and on adjacent areas of the mantle in midventral regions where alone much light can enter. The middle, sensory lobe of the mantle edge carries tentacles (*t*) and eyes, of which the specimen shown in figure 3 had 63—27 on each free surface with 3 in the region where the middle lobes are fused anteriorly and 6 in the more extensive region of posterior fusion (*p*).

CILIARY CURRENTS

The ciliary currents in the mantle cavity of *Pecten irradians* and of *P. tenuicostatus* have been described by Kellogg (1915). The ctenidia are plicated with broad principal filaments, on the surface of which particles are conducted away from the free margin of the demibranchs, whereas the frontal cilia on the ordinary filaments composing the intervening ridges carry material toward these margins. Precisely the same conditions prevail in *Hinnites*. Mucus-laden food streams are conveyed oralward in the food groove along the free margins of the demibranchs and also along the axes and the edges of the reflected filaments (see arrows in fig. 3). Kellogg has stressed the significance of the different direction of beat of the frontal cilia on the two types of filaments. Larger particles and mucus-laden masses are carried on the surface of the ridges composed of ordinary filaments to the food grooves in the free margins of the demibranchs. They tend to fall from these grooves onto the mantle surface and so to be rejected. Smaller collections may penetrate between the ridges and so be carried by the frontal cilia on the principal filaments either to the axis or to the edges of the reflected filaments according to whether they are on the inner or outer faces, respectively, of the demibranchs. By either of these latter routes they will pass oralward and always reach, at least, the palps, from which they may or may not be further conveyed to the mouth.

The rejection currents on the visceral mass are shown in figure 4. From the greater part of the surface particles are carried to the base of the foot, whence they pass on to the right mantle lobe below. Over the posterior quarter of the surface the currents beat backward and particles are carried to the extreme tip

of the visceral mass. In unattached species of *Pecten* and allied genera the currents on the mantle surface are powerful, and both in front of and behind the adductor muscle waste material is carried dorsally into ciliary vortices where it is accumulated into pseudofaeces (Kellogg, 1915). As a result of sudden contractions of the adductor these masses are from time to time expelled on either side of the hinge line, in an anterodorsal and a posterodorsal direction. This dorsally directed rejection of waste prevents any disturbance of the water from which the inhalant current is drawn, and—but only incidentally—makes possible the swimming

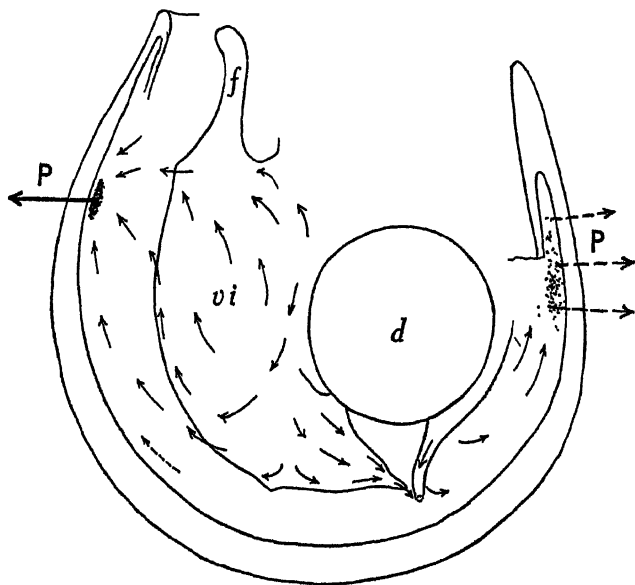


Fig. 4. *H. multirugosus*, same specimen as fig. 3 after removal of etendia, showing course of ciliary currents on visceral mass (*vi*) and on right mantle lobe. Pseudofaeces collect in compact mass anteriorly and over wider area posteriorly, direction of expulsion shown by large arrows (*P*). $\times 1$.

movements exhibited in these species (Yonge, 1936). In *Hinnites* the rejection currents on the surface of the mantle are much weaker. As shown in figure 4, there are relatively strong ciliary currents anteriorly and large masses of pseudofaeces accumulate within the end of the mantle opposite the base of the foot. There is also some accumulation posteriorly but the currents here are much weaker and the waste material is not actually massed together as it is anteriorly. The main agent of expulsion on this side is the very powerful exhalant current. This emerges from between the valves in a posterior rather than in a dorsal direction (a consequence of attachment of the shell to the substratum) as indicated in figure 5. Only from the anterior half of the mantle cavity is waste matter removed by periodic contractions of the adductor. Material from the greater part of the surface of the visceral mass is eventually carried in an anterior direction; that from its posterior surface and from the surface of the rectum is conveyed posteriorly. Midventrally, ciliary currents on the mantle surface are extremely weak or nonexistent. In *Pecten* they are powerful (Kellogg, 1915) and the difference in *Hinnites* may be

connected with the different habitat—attached to rocks where water movements are very great and there is little sediment.

MODIFICATIONS ASSOCIATED WITH ATTACHMENT

The greater length of the dorsoventral diameter in *Hinnites*, compared with the unattached species, is shared with byssal-attached species. It is probably primitive for the Pectinidae. Increase in the length of the anteroposterior diameter appears to be associated with freedom and increased efficiency in swimming; this axis is the greater in unattached species of both *Pecten* and *Chlamys*, while in *Amusium* the two diameters are practically equal. In *Hinnites*, details of shape, rather than proportions, alter after attachment, but the etenidia are now disposed somewhat differently, the tips of the adult etenidia being directed ventrally so that the exhalant current flows out in a posterior direction (fig. 5).

The features of major interest in the attached shell are the hinge and the ligament. In the young shell there is a straight hinge line without teeth but with a thin external ligament and a centrally placed and very condensed elastic inner ligament or resilium, exactly, that is, as in *Pecten* or *Chlamys*. After attachment the hinge line gradually ceases to be straight, it tends to bend ventralward at both ends although usually to a greater degree posteriorly. It may also become somewhat irregular in the horizontal plane. With further growth the outline of the auricles becomes obliterated. Most important of all, the hinge line may be displaced ventrally as a result of the movement of the mantle in that direction. This does not always occur and where it does, the displacement varies greatly in different animals. This would appear to be a direct effect of environmental factors on the growing animal.

Where *Hinnites* becomes attached in regions where growth can continue without restraint the hinge line is unaffected. This was true of several specimens collected from a concrete storage tank in which they had become cemented and were under no constraint. In nature, however, the young tend to attach themselves, initially by byssus threads and finally by cementation, in the shelter of cracks and crevices where there is protection from the violent Pacific surf but corre-

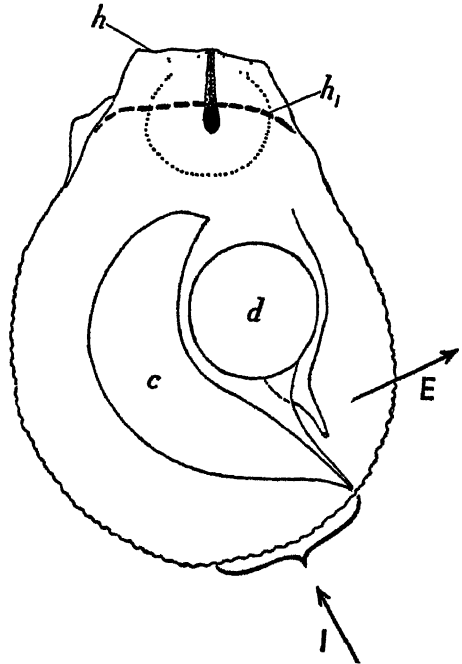


Fig. 5. *H. multirugosus*, attached, right valve showing typical disposition of etenidia (c) in life with inhalant current (I) restricted to area indicated by bracket where alone pallial curtains not in contact. E, exhalant current directed posteriorly; h, h₁, original and final hinge lines (latter indicated by broken line). Area of right shell valve at time of attachment indicated by dotted outline. $\times \frac{1}{2}$.

spondingly restricted space for subsequent growth. Under such circumstances, as indicated diagrammatically in figure 6, death owing to inability to open the valves would be the result of growth if the hinge line remained in its original position. But under such circumstances the mantle tissues must react by migrating ventralward with consequent formation of new hinge lines one after another, so long, presumably, as the stimulus acts. This leads to the condition shown in figure 3. Of the total dorsoventral diameter (9.4 cm.) of the animal there shown no less than 20 per cent (1.9 cm.) lies dorsal to the functional hinge line. The successive lines occupied during growth by the elongated external ligament, together with

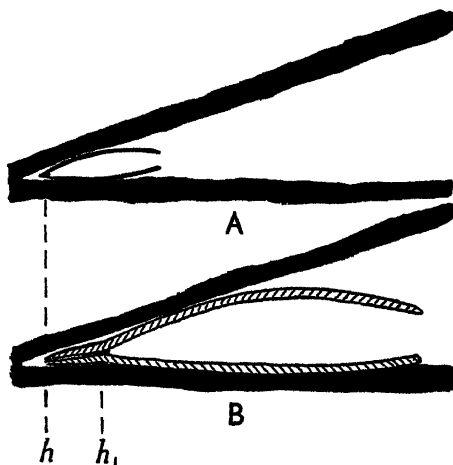


Fig. 6. *H. multirugosus*, diagrams showing effect of ventralward migration of hinge line. A, attachment in crevice of young specimen, hinge line at h ; B, after considerable growth, hinge line moved to h_1 so that valves can continue to open.

the stimulus of constraint, of moving the position of the hinge line. Site of attachment and possible migration of the hinge line may be regarded as joint adaptations to life in extremely turbulent waters.

DISCUSSION

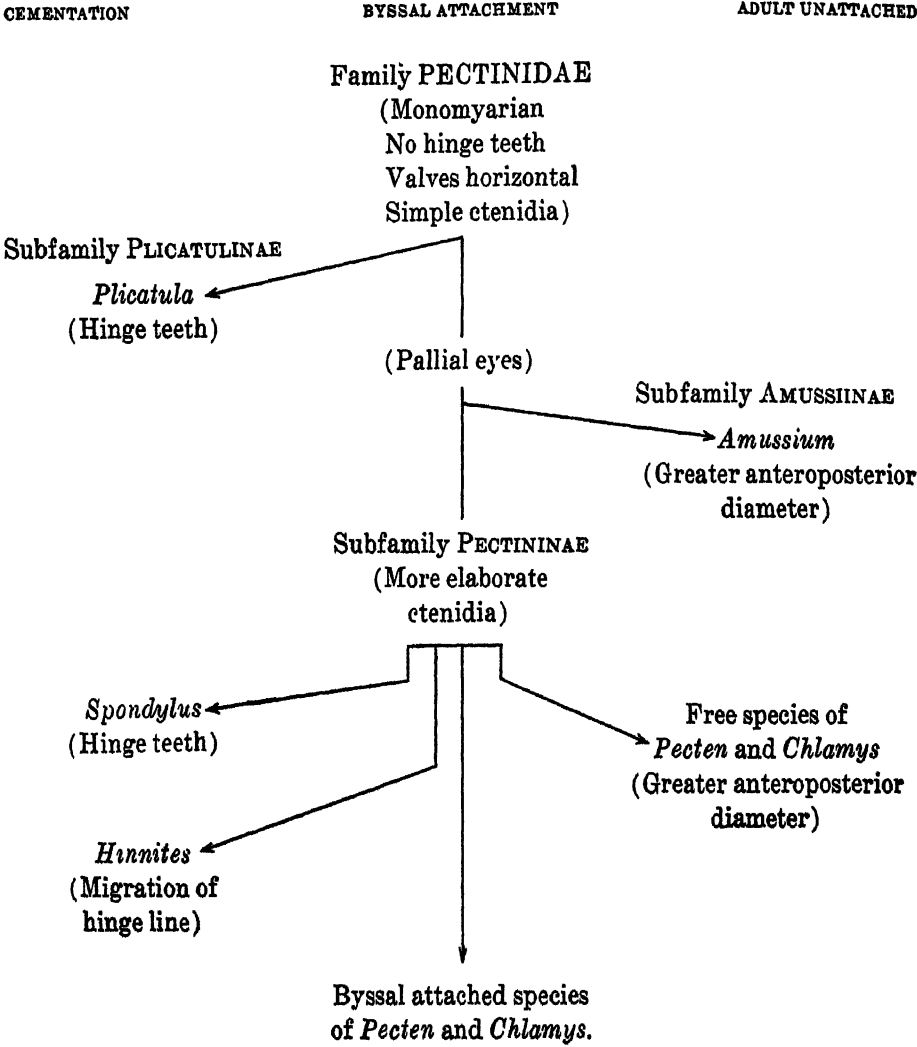
It is instructive to compare conditions in *Hinnites* with those in related genera which also are cemented. Jackson (1890) considered that *Pecten*, *Hinnites*, *Spondylus*, and *Plicatula* might be serially connected in that order. The cementation of a partly grown *Pecten* would lead to the condition found in *Hinnites*. In *Spondylus* he found evidence of a byssal notch in the first postlarval, termed by him "nepionic," shell. This would indicate a period of byssal attachment prior to cementation, although cementation clearly occurs much earlier in life than in *Hinnites*. In *Plicatula* he found no trace of a byssal notch and attachment presumably occurs, as in *Ostrea*, at the end of the prodissoconch stage. On the basis of these observations, Jackson postulated that *Spondylus* might have passed through an "Hinnites" stage in phylogeny and *Plicatula* through this and also a "Spondylus" stage.

the pit and the remains of the formerly functional areas of the internal ligament within this, are all clearly shown in this region. In consequence of this reaction, presumably to constraint between rock surfaces when the upper valve is raised, *Hinnites* is able to live and to grow, often to a considerable size, after having settled in a region that provides the initial advantage of maximum protection. It seems reasonable to regard the form of behavior which leads the animal to settle in protected crevices as an essential adaptation to life between tide marks on very exposed coasts. The young of other Lamellibranchia have similar habits, e.g., *Hiatella* (Hunter, 1949). But this adaptation would often be of little use without the added capacity, evoked by

and *Pecten* (and so *Hinnites*). But the presence in *Spondylus* of secondary hinge teeth and the mode of cementation by way of the spines indicate a separate line of evolution. This is still more true of *Plicatula*, in which the gills are simpler as they are also in *Amusium* (Ridewood, 1903), the pallial curtains are small, there are no pallial eyes with a possibly associated simplicity in the visceral ganglia, the foot is reduced to a slight ridge, and there is an increase in the dorsoventral diameter with ventralward migration of the adductor (Watson, 1930). Available evidence suggests that *Plicatula* branched off before the pectinid gill attained its present complexity and possibly before pallial eyes were evolved. (If they have been retained after cementation in *Spondylus* why not in *Plicatula*?) Without going further into the matter the available evidence suggests independent assumption of cementation by *Plicatula*, then by *Spondylus*, and finally by *Hinnites*, as indicated in the accompanying table. There seems no reason however for assuming "Hinnites" and "Spondylus" stages in the evolution of cementation.

Interest centers in the existence of three types of cementation and the possible reasons for this. As previously discussed (Yonge, 1936), the evolution of the family Pectinidae (see Thiele, 1935, for details of classification) with regard to byssal attachment has led to orientation of the animals with the valves lying horizontally instead of vertically. It is with valves so disposed that the free species swim (unlike *Lima* which swims with the valves held vertically). But all Pectinidae, except perhaps *Plicatula* (subfamily Plicatulinae), are attached by a byssus in early life and many of them throughout life. From this condition, as shown in the table, there are possibilities of evolution in two directions: adult freedom, separately acquired at least twice, namely by *Amusium* (subfamily Amussiinae) and by certain species of *Pecten* and *Chlamys* (subfamily Pectininae), and cementation. Possibilities for the latter are obviously presented by the normal position with one valve lying against the substratum (always of rock, to permit byssal attachment), and cementation has certainly come about on three separate occasions. It is particularly interesting, however, to find that similar changes in habit have occurred at different stages in the life history, namely, early postlarval (*Plicatula*), late postlarval (*Spondylus*), and after the shell has assumed the adult form (*Hinnites*). There seems no reason for assuming that the original change was made at later stages in life than it is at present. The general conclusion reached is that similar possibilities presented to the same stock may produce a series of similar responses, but not necessarily each time in precisely the same manner or at the same stage in the life history. It is interesting to note that cementation in all three groups has affected the hinge, by the secondary appearance of hinge teeth of distinct types in *Plicatula* and *Spondylus*, and by the capacity for ventralward movement in *Hinnites*. Freedom from attachment seems to have the effect of increasing the anteroposterior diameter. Meanwhile the original habit, byssal attachment, is maintained by a significant proportion of the Pectininae. The substance of these speculations in phylogeny is contained in the evolutionary tree given in the accompanying table.

EVOLUTIONARY TREE FOR PECTINIDAE



SUMMARY

Hinnites multirugosus is a pectinid lamellibranch which becomes cemented to the substratum at an unusually late stage in growth. Previous to this it is usually attached by a byssus but is capable of the swimming and other movements executed by unattached pectinids. Size at final attachment varies greatly—certainly between 2.2 and 4.2 cm. in dorsoventral diameter. Attachment is always by way of the right valve and later growth is irregular.

Adult structure varies little from that of unattached species of *Pecten* or *Chlamys*, the greater dorsoventral diameter in *Hinnites* being probably primitive and the greater anteroposterior diameter in the free species secondary. The ctenidia in adult *Hinnites* are so disposed that the exhalant current emerges in a posterior instead of a dorsal direction. Cleansing currents are very weak in the ventral and posterior regions of the mantle cavity, probably in correlation with life in turbulent water with little suspended matter.

In adaptation to the dangers of the exposed habitat, intertidal or in shallow water along the exposed Pacific coast, the young tend to move into the shelter of narrow crevices and there eventually become cemented. Subsequent ventralward migration of the hinge line is possible and this occurs where restriction of space would prevent the growing shell valves from opening. There is apparently no such movement of the hinge line where there is no constraint.

The primitive habit of members of the family Pectinidae must certainly have been byssal attachment and this is retained throughout life by many species in the subfamily Pectininae. But there is the possibility of departure from this, on the one hand by cementation and on the other by freedom. The former has occurred on three distinct occasions, in *Plicatula* in the Plicatulinae, and in *Spondylus* and *Hinnites* in the Pectininae. In each attachment takes place at a different stage in the life history. Freedom from byssal attachment has been attained independently at least twice, in *Amussium* in the subfamily Amussiinae and in species of *Pecten* and *Chlamys* in the Pectininae. In every case, with the probable exception of *Plicatula*, a period of byssal attachment precedes eventual cementation or freedom.

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STUDIES ON
PACIFIC COAST MOLLUSKS

IV. OBSERVATIONS ON SILIQUA
PATULA DIXON AND ON EVOLUTION
WITHIN THE SOLENIDAE

BY
C. M. YONGE

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OBSERVATIONS ON SILIQUA PATULA DIXON AND ON EVOLUTION WITHIN THE SOLENIDAE

BY

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University of Glasgow

INTRODUCTION

THE RAZOR CLAM, *Siliqua patula*, is the commonest representative of the Solenidae along the Pacific coast of North America, ranging from Monterey to Alaska (Weymouth, 1920; Keen, 1937). It is economically an important species. Its occurrence, on exposed sandy beaches, has been described by Weymouth (1920), Fraser (1930), and Quayle (1941). In common with other species of the Solenidae, *S. patula* is highly adapted for life in this exposed and unstable environment owing to its capacity for rapid vertical burrowing into sand, as experimentally studied and described by Fraenkel (1927) for species of the still more specialized *Solen* and *Ensis*. Adaptation for rapid vertical movement through sand involves the form of shell—its elongation and lateral compression—and the form and disposition of the greatly enlarged foot. *S. patula* is of particular interest because, although it exhibits all the essential characters of the Solenidae, it is less highly modified than are species of *Ensis*, *Solen*, or *Cultellus* with their more elongate, narrower, and almost semicylindrical shell valves.

This paper is initially concerned with observations on the structure, ciliary currents, and major adaptations of *Siliqua patula*, which for so common and economically so important a species has received very little previous attention. Further, by comparison with the structure of *Ensis arcuatus*, evidence is presented of the probable course that evolution has taken within the Solenidae, one of the most highly specialized families of the Lamellibranchia.

The observations on living *Siliqua patula* were made at the Hopkins Marine Station, Pacific Grove, California, during the summer of 1949, specimens being collected at Ocean Beach, Elkhorn Slough. Comparative data on *Ensis arcuatus* (Jeffreys), largely confirming the work of Graham (1931), were obtained at the Millport Marine Laboratory, Scotland. Thanks are due to Dr. D. B. Quayle for sending additional supplies of preserved *S. patula* from Vancouver Island, and to Dr. H. F. Steedman and Mr. G. Owen of the Department of Zoölogy, University of Glasgow, for the preparation of slides and sections.

SILIQUA PATULA

Shell.—As shown in figure 6, B, the shell is oval, about three times as long as it is broad, and laterally compressed, being about twice as broad as it is thick. The umbo is situated approximately one-third of the distance from the anterior end. The hinge dentition is typical of the Solenidae, with three cardinal teeth on the left valve and two on the right. The well-developed external ligament extends for some distance posteriorly. The shell valves are thin, but from the region of the hinge a thickened rib extends ventralward with a slight anterior inclination. It forms a ridge between the insertions, anterior and posterior to it, respectively, of

the anterior adductor and the anterior pedal retractor. For reasons to be given later, this rib may be functionally associated with the latter muscle.

Mantle.—Even when the adductors are fully contracted, the margins of the shell valves are not in contact anteriorly, ventrally, or posteriorly. As shown in figure 1, the mantle tissues exposed between the shell valves are protected by a

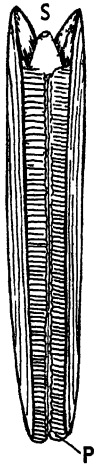


Fig. 1. *Siliqua patula*, ventral aspect, animal fully withdrawn. $\times 1$. P, periostracum; S, retracted siphons.

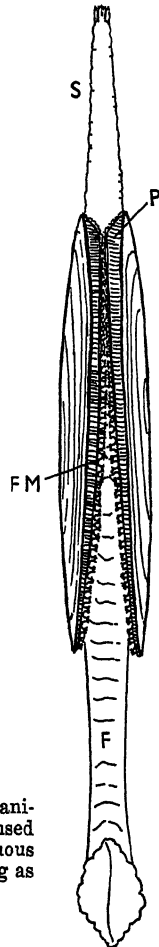


Fig. 2. *Siliqua patula*, ventral aspect, animal fully distended. $\times 1$. F, foot; FM, fused mantle edges (inner lobes only) continuous with siphons (all stippled). Other lettering as in fig. 1.

thick periostracum. This is possible because the periostracal groove is separated by some distance from the outer lobe of the mantle edge, which secretes the calcareous margin of the shell. When the shell valves close, the periostracal groove is pulled inward, over the middle and inner lobes of the mantle edge, in such a way that only mantle tissue covered with periostracum is exposed. Posteriorly the gape between shell valves is greater so as to accommodate the large siphons, the retracted tips of which lie just within the posterior margins of the shell valves (fig. 1).

The appearance, viewed from the same aspect as in figure 1, of an animal with both siphons and foot extended is shown in figure 2. The pedal gape is large (figs. 2 and 3) and is fringed by a row of conspicuous dendritic tentacles which are

borne on the middle (sensory) lobe (ML) of the mantle edge (Yonge, 1948a). The inner (muscular) mantle lobe (IL) is less developed and consists of a smooth ridge without tentacles. This lobe of the mantle edge fuses with its fellow on the opposite side rather less than halfway along the ventral surface to form the posterior boundary of the pedal gape. Fusion is complete; the tissues on the two sides unite and a broad band of muscle extends between the shell valves. This region of fusion (FM) is continued posteriorly to form the siphons (fig. 2). The middle mantle lobes, with the tentacles rapidly diminishing in size posteriorly (fig. 2), and the periostracal grooves of the two sides do not fuse, but, as shown in figures 2 and 3, continue round the base of the siphons.

Siphons.—The siphons, as already noted, are formed from the fused inner mantle lobes exclusively, and thus have no protective covering of periostracum as, for instance, in the Myacea. They are fused, except terminally, and are relatively long, extending for a distance exceeding one-third of the length of the shell. In life they are brown in color with a fine surface annulation, except terminally where the separate openings are smooth and white. There is a common ring of about fifteen rather scattered outer tentacles. The inhalant aperture is guarded by three rows of tentacles consisting of inner and middle rows, each of six pinnate tentacles, and a third, outer row of twelve simple tentacles. All bend inward and, the members of succeeding rows alternating, they form a very efficient straining system similar to that in *Platyodon cancellatus* (Yonge, 1951). The exhalant aperture consists of a tapering tubular membrane with a further series of about ten small tentacles between the aperture and the common outer ring.

Foot.—As in all the Solenidae, the foot is most highly developed; it is long and in section oval. As shown in figure 2, it can extend forward for a length approximately equal to that of the shell. It functions in the same manner as the foot of other Solenidae studied by Fraenkel (1927). The typical movements of a bivalve burrowing into a soft substratum are first diagonally down and then horizontally forward, for example as described by Quayle (1949) for *Venerupis* (*Paphia*) *pullastra*. In *Siliqua*, although the considerable extension of the pedal gape along the ventral surface still permits the foot to be extruded in an anteroventral direction (see fig. 7, B), final extension is anterior, in the long axis of the shell, and movement is vertical. The great length and power of the foot ensures, as in all Solenidae, deep withdrawal of the animal after each successive extension of the foot. As in *Ensis siliqua* (Graham, 1931), there is a large byssus gland in young individuals of *S. patula*.

The animal is pulled downward by the contraction of the two pairs of pedal retractors (AR, PR), which are inserted, as always, near the dorsal margins of the shell valves and just within the adductors. This position of attachment is very efficient when the result of contraction is to pull the bivalve into (or against) the substratum with the ventral, or anteroventral, shell margins undermost. But the shell valves must be exposed to considerable strain if the anterior ends of the valves are undermost, because the insertions of the pedal retractors will then be in line and all the pull will be exerted along the dorsal margin of the shell. The presence of the strengthening rib, to the hinder surface of which the anterior (and larger) pedal retractor is inserted, appears to solve this difficulty.

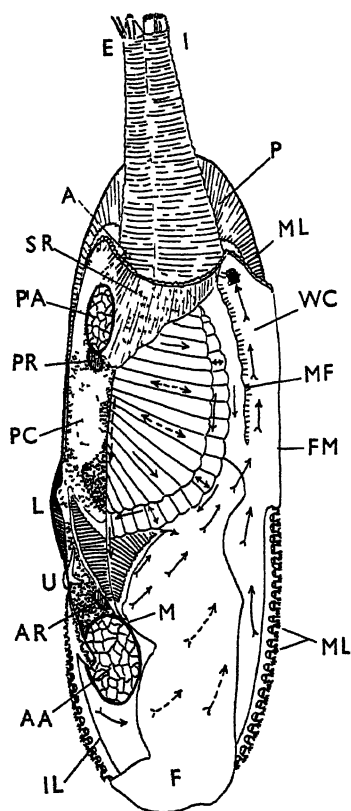


Fig. 3. *Siligua patula*, mantle cavity viewed from left side after removal of left shell valve and mantle lobe. $\times 1\frac{1}{2}$. A, anus; AA, anterior adductor; AR, anterior pedal retractor; E, I, exhalant and inhalant siphons; IL, inner lobe of mantle edge; L, ligament; M, mouth; MF, mantle fold; ML, middle lobe of mantle edge (with tentacles around pedal gape); PA, posterior adductor; PC, pericardium; PR, posterior pedal retractor; SR, siphonal retractor; U, umbo; WC, waste canal. Other lettering as before. Plain arrows indicate feeding currents on exposed surfaces; broken arrows, those on undersurfaces; feathered arrows, cleansing (i.e., rejection) currents; where broken, those on mantle surface where obscured by foot.

Mantle cavity.—The disposition of the organs in the mantle cavity is shown in figure 3. The adductor muscles are elongated; the anterior muscle is about twice as large as the posterior one. The great size of the anteriorly directed foot causes displacement of the visceral mass and ctenidia to the posterior third of the mantle cavity, but both remain large and are little modified in form. The ctenidia are plicate and heterorhabdic, as in *Ensis* and *Solen* (Ridewood, 1903; Atkins, 1936, 1937a); the inner demibranchs are appreciably larger than the outer ones. The palps are well developed. On the mantle surface, below the ctenidia in the region of ventral mantle fusion, a pair of folds (MF) project horizontally and enclose, beneath their applied summits, a ventral waste canal (WC).

Feeding currents.—On the ctenidia (fig. 5, A) the frontal cilia on all filaments on both the outer surface of the outer demibranchs and the inner surface of the inner demibranchs carry particles exclusively to the free ventral margins, which are grooved. On the opposed faces of the demibranchs the frontal ciliation is different on the principal and ordinary filaments. On the latter, small frontal cilia carry finer particles to the ctenidial axis and large cilia produce currents in the opposite direction. In these, larger particles or mucus-laden masses are conveyed to the free margin of the demibranchs. On the principal filaments only the larger frontal cilia appear to be present because all cilia beat ventralward. There are three oralward currents on each ctenidium, one along the axis dorsally and the other two along the free margins of the demibranchs ventrally (see fig. 5). Comparison will later be made with conditions described by Atkins (1936, 1937a) in

Solen, *Ensis*, and *Cultellus*, but it may be noted now that the extensive surface of lateral cilia on the ctenidia in *S. patula* causes a powerful inhalant current, with the result that considerable quantities of suspended matter must enter. These are rigorously sorted, and only the finest particles, which are carried dorsalward to the ctenidial axes and then oralward, are likely to reach the mouth. Larger particles and masses which are moved forward in the marginal grooves are likely to be transferred to the surface of the mantle or, if they do reach the palps, to be rejected

Disposal of waste.—The mantle surface is generally ciliated anteriorly, but posteriorly cilia are confined to the waste canal, including the ventral surfaces and summits of the bounding mantle folds. The basal regions of the foot, where this merges with the visceral mass, are also ciliated. As shown in figure 3, all waste particles on the mantle surface, from the visceral mass and from the tips of the palps, are carried posteriorly (topographically upward when the animals are in their normal posture in sand) and are directed ventrally into the waste canal. Cilia within this canal create a powerful current in which material is carried to the base of the inhalant siphon. There it accumulates to form masses of pseudofaeces which are periodically expelled through this siphon.

Mantle folds.—This would appear to be the first record of the presence of mantle folds in any species of the Solenidae; they do not occur in species of *Solen*, *Ensis*, or *Cultellus*. Similar folds have been described in the Mactridae: in species of *Mactra* and *Schizothaerus* (Kellogg, 1915), of *Spisula* (Kellogg, 1915; Yonge, 1948b), and of *Lutraria* (Yonge, 1948b); also in certain of the Tellinacea: in species of *Macoma* (Kellogg, 1915; Yonge, 1949), *Tellina*, *Scrobicularia*, and *Abra* (Yonge, 1949). In the Mactridae the presence of mantle folds appears to be correlated initially with that of a siphonal membrane which directs the inhalant current ventralward and thus clear of the gills when it enters the mantle cavity. This is probably an adaptation for life in silty sand (Kellogg, 1915). In *Lutraria*, however, the folds have been retained after this siphonal membrane has been lost, and serve to direct waste into the fourth pallial aperture for extrusion (Yonge, 1948b). In the deposit-feeding Tellinacea the presence of mantle folds enables their possessors to continue drawing in great quantities of bottom material without interfering with the accumulation of great masses of pseudofaeces which is going on at the same time (Yonge, 1949). In *Siliqua patula*, which lives in very firm sand, never in silty sand, and has no such siphonal flap, the former explanation of the presence of these folds cannot apply; nor do conditions of food intake in any way resemble those of the deposit-feeding Tellinacea.

S. patula has a different problem, that of disposing, through the inhalant siphon, of the quantities of sand which certainly enter the mantle cavity through the extensive pedal gape while the animal is burrowing. The action of the gills would be affected, and their structure possibly damaged, if this sand was forced against them, especially when the foot was drawn back. But the presence of the mantle folds confines the sand to the ventral region of the mantle cavity, and the waste channel so formed probably increases the efficiency of discharge through the long inhalant siphon when the adductors contract. This suggestion in regard to the function of the mantle folds in *S. patula* is supported by the fact that these structures are absent in the more specialized species of *Solen*, *Ensis*, and *Cultellus*, in all of which the pedal gape is much smaller, being restricted to the anterior end, while sand grains are almost entirely excluded by the presence of flanking folds of the inner mantle lobe which form the pedal valve (fig. 4, PV).

ENSIS ARCUATUS

Ensis arcuatus, a species easily obtainable in Fairlie Sands near Millport, Scotland, was chosen for comparison with *Siliqua patula* as being a typical example of the

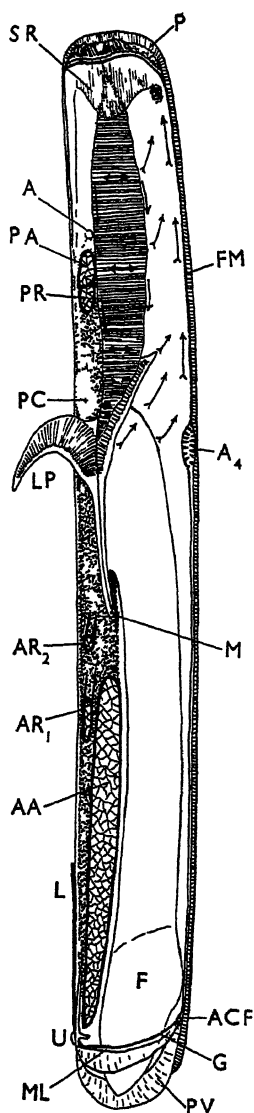


Fig. 4. *Ensis arcuatus*, mantle cavity viewed from left side after removal of left shell valve and mantle lobe. $\times 1$. A_4 , fourth pallial aperture; ACF, anterior end of region of cuticular fusion of mantle lobes; AR_1 , AR_2 , two insertions of anterior pedal retractor; G, periostracal groove; LP, labial palp; PV, pedal valve (enlarged inner lobe of mantle edge). Other lettering, and arrows, as in fig. 3.

more highly modified razor shells of the genera *Solen*, *Ensis*, and *Cultellus*. Aspects of both the structure and the physiology of species of *Ensis* and *Solen* have been given by Fraenkel (1927), Graham (1931), and Atkins (1936, 1937a, 1937b); Holme (1951) has recently described the characters which distinguish the three British species of *Ensis*.

The major differences between *E. arcuatus* and *S. patula* are best indicated by reference to figures 3 and 4. The shell is greatly elongated, about eight times longer than it is broad, with dorsal and ventral margins parallel—both, in *E. arcuatus*, being slightly curved. The valves gape anteriorly and posteriorly but not ventrally. Each has almost the form of a half cylinder, so that the animal is nearly circular in cross section—the thickness being almost equal to the breadth—except posteriorly, where it is somewhat compressed laterally. The umbo (U) lies at the extreme anterior end, in consequence of which the anterior cardinal and lateral teeth in each valve have been lost. The ligament (L) is greatly extended, uniting the anterior fifth of the dorsal margins of the shell valves. Posterior to this the valves are separate, but are closely attached by thick periostracum to the mantle edge. The two periostracal grooves are very near one another, the intervening regions of mantle edge being fused. In effect, therefore, the shell valves are intimately connected along their entire length dorsally.

Ventrally the mantle edges are united for most of their length, but there are interesting differences from *S. patula*. Along the posterior third there is complete tissue fusion of the inner lobes of the mantle edges, the united tissues (FM) being thick and muscular. Union along the remainder of the ventral surface, which extends to the anterior end of this (ACF), is by cuticular fusion of the inner mantle lobes, as described by Atkins (1937b). Between these two regions of fusion, opposite the base of the foot, is a small fourth pallial aperture (A_4). This is the only region where the middle mantle lobes are sufficiently developed to carry tentacles, the arrangement of which assists in distinguishing the different British species of *Ensis* (Holme, 1951). A fourth pallial aperture is present also in *Cultellus* but not in *Solen* (Bloomer, 1903; Atkins, 1937b). Morphologi-

cally it represents, with the region of cuticular fusion, a part of the pedal gape (Bloomer, 1903), but functionally the latter opening is restricted to the anterior end. Cuticular fusion is certainly of more recent origin and of much less strength than the tissue fusion posteriorly. The anterior mantle edges readily separate, thus uniting the pedal and fourth pallial apertures, when animals become moribund (Atkins, 1937b). But in life, with the pressure of the substratum assisting in holding the shell valves together, the mantle lobes are always firmly united in this region.

The pedal gape is bounded by prominent lips formed by the great local hypertrophy of the inner (muscular) lobes of the mantle edge and constituting the pedal valve (PV) (Graham, 1931), but the middle mantle lobe (ML) is reduced to an insignificant ridge that lies very close to the periostracal groove (G). The relative degrees of development of these two lobes are thus precisely the opposite of what they are in *S. patula*.

The siphons are very short and, although shown contracted in figure 4, when fully expanded they project only a very short distance beyond the posterior margin of the shell (see fig. 8, B). Precisely as in *S. patula*, they are formed exclusively by fusion of the inner lobes of the mantle edge. Both inner and outer rows of tentacles are more numerous than in *Siliqua* [see Morse (1919) for description and figure of those in *Solen* (= *Ensis*) *ensis*], but all are simple and there is here no elaborate system of straining tentacles.

Within the mantle cavity the most obvious difference from *S. patula* is the much greater enlargement of the foot, the base of which extends so far back that the visceral mass and gills are restricted to the posterior third of the mantle cavity. The foot is almost circular in section and, owing to the cuticular fusion of the mantle lobes ventrally, it can be extruded only forward (i.e., in life, normally downward). The anterior adductor is greatly extended and many times larger than the posterior muscle. Posteriorly there is one pedal retractor, but anteriorly there are two insertions (AR_1 , AR_2), a result presumably of the elongation of the body. The same condition is found in *Cultellus* (Graham, 1934). As well as being displaced to the posterior end, the ctenidia are reduced in depth, so that the effective feeding and respiratory surface is relatively much less than in *S. patula*. But in detailed structure the ctenidia of the two species are very similar. Atkins (1936, 1937a) has described their ciliation and also that of ctenidia of *Cultellus pellucidus*, which differ structurally in being nonplicate and homorhabdic.

In figure 5, comparisons are made between the currents on the frontal surfaces of the ctenidia in A, *S. patula*, B, *Solen* and *Ensis* spp., and C, *Cultellus*. The effect of the different ciliation on the principal filaments present in the plicate gills of A and B is also shown. The differences revealed are interesting and appear to be correlated with the mode of life. *S. patula* lives in agitated waters; species of *Ensis* and *Solen* generally occur in beaches less fully exposed to violent oceanic surf. *C. pellucidus* lives at moderate depths on a mud bottom, where water movements are much less. In *S. patula* much suspended matter must enter, despite preliminary straining by the tentacles of the inhalant siphon; in species of *Solen* and *Ensis* less will probably enter (certainly in *E. arcuatus*), and less again in *Cultellus*. Accordingly, greater selective activity is required of the ctenidia of *S. patula*, less for

E. arcuatus, and still less for *Cultellus*. Generally speaking, the material which is carried forward by dorsal streams is much more likely to reach the mouth than that which passes along the rather inappropriately named "food" grooves along the ventral margins of the demibranchs. Whenever much material enters the mantle cavity the greater part is carried into these ventral channels from which it is soon transferred to the walls of the mantle cavity. It is noteworthy that the ratio of dorsal to ventral ciliary streams in *S. patula* is 1:2; in *Ensis* and *Solen*, 3:2; in

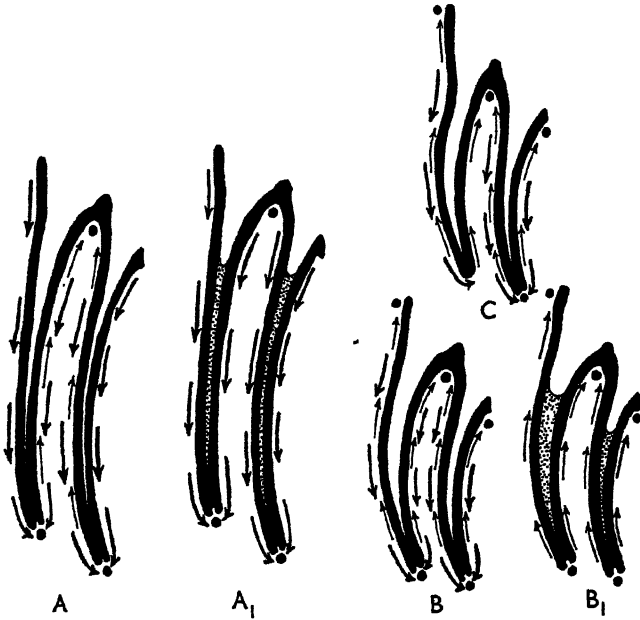


Fig. 5. Diagrams of frontal ciliation. *Siliqua patula*, A, ordinary filament; A₁, principal filament. *Ensis* or *Solen*, B, ordinary filament; B₁, principal filament. *Cultellus*, C, all filaments (gill not plicate). Large arrows denote currents caused by large frontal cilia; small arrows, those caused by small frontal cilia; dark circles, oralward currents. (B, B₁, C, after Atkins.)

Cultellus, 3:1. The addition of two dorsal streams, at the extremities of the "ascending" filaments as well as along the axis, in B and C is striking; so is the loss of one ventral groove in C. Moreover, in A, food collection is confined mostly to the opposed faces of the demibranchs, while on the principal filaments (A₁) all frontal cilia beat ventralward; in B, on the other hand, food can effectively be collected by all faces of the demibranchs, while on the principal filaments (B₁) cilia beat exclusively toward the dorsal side.

The major difference in mantle ciliation is the absence in *Ensis*, as noted already by Graham (1931), of cilia anterior to the base of the foot. This region is presumably kept clear of fragments by the piston-like movements of the foot. There are no mantle folds, but cilia on the posterior mantle walls carry material into the ventral channel for rejection through the inhalant siphon.

The presence of the fourth pallial aperture may also be associated with the hypertrophy of the foot. Various views have been expressed about the function of this aperture (Bloomer, 1903; Atkins, 1937b), but further examination has only con-

firmed the previous opinion (Yonge, 1938b) that it acts as a safety valve through which some of the water in the mantle cavity is expelled when the pedal retractors, and also probably the adductors, contract. This involves withdrawal of the foot into the mantle cavity, the pedal valve sliding down the sides of the stationary and dilating foot, and hence displacement of water posteriorly. Although much of this passes out through the inhalant siphon, some additional outlet seems necessary to prevent splitting the mantle lobes where they are united only by cuticular fusion.

This additional extent of mantle fusion increases efficiency by retaining the elongated foot within the closed cylinder and thus restricting its movements to those in the long axis of the shell and body. At the same time, elongation of the shell has the effect of reducing the ratio of transverse sectional area to volume. This, in the most convenient terms of square millimeters to cubic centimeters, is about 10 : 1 in *Siliqua patula* compared with 7 : 1 in *Ensis arcuatus*. Another result of the reduction in breadth of the shell is to make the insertions of the pedal retractors more effective in relation to the exclusively anteroposterior movements of the foot.

Posterior extension of the shell has also been associated with reduced length of the siphons, which can now be more easily withdrawn into the shelter of the shell valves. This is the more important because the siphons in the Solenidae are not protected by periostracum as they are in other deep burrowers, e.g., *Mya* (Myidae), *Lutraria* (Mactridae), and *Panope* (Hiattellidae), in all of which the periostracal groove is included in the tissues fused to form the siphons. The long, unprotected siphons of *S. patula* would seem to be somewhat of a liability. This danger is overcome in species of *Solen*, *Ensis*, and *Cultellus*. These are among the most highly specialized of Lamellibranchia, with striking powers of rapid vertical movement through soft, typically unstable, substrata.

EVOLUTION WITHIN THE SOLENIDAE

Discussion of the foregoing accounts of structure in *Siliqua* and *Ensis* may suitably deal with the possible course of evolution within the Solenidae. Certain points require initial emphasis. There is the fundamental, though largely unrecognized, fact that throughout the Mollusca the growth of the body and the growth of the shell must be considered separately. To the bilaterally symmetrical body of the primitive mollusk with its anteroposterior axis of growth was attached, as it were, a rounded or oval covering of tissue which increased peripherally. In the Lamellibranchia this mantle and the calcareous shell it secretes have become laterally compressed and bilobed and entirely enclose the animal. The head has been lost and the collection of food has been transferred to the ctenidia. The possible course of evolutionary change, involving a stage similar to that still retained in the Nuculidae, has been discussed elsewhere (Yonge, 1939). The final effect, however, is that the bilaterally symmetrical body with its anteroposterior axis of growth is now contained within mantle lobes secreting shell valves which grow by marginal increments. In the simplest case, where the shell is circular with "transverse" and vertical axes of the same length, exemplified by *Glycymeris* (figs. 6, A, 7, A), growth is greatest in the region opposite the umbo, decreasing symmetrically round both margins. Hence, as shown by D'Arcy Thompson (1942), to whom reference should be made for mathematical treatment of the problem, lines of growth will be laid down as a series of

coaxial circles each of which, as indicated in figure 6, A, passes through a point very close to, though not actually coinciding with, the umbo. Since the umbones are dorsal, with the connecting ligament between them representing the uncalcified mid-dorsal region of the shell, the region of greatest growth is mid-ventral. Here the dorsoventral vertical (or median) axis also represents the major growth axis of the mantle. It is thus at right angles to the anteroposterior axis of the body, which may be represented by a line connecting mouth and anus or, perhaps more accurately where both muscles occur, by one which runs through the center of each adductor.

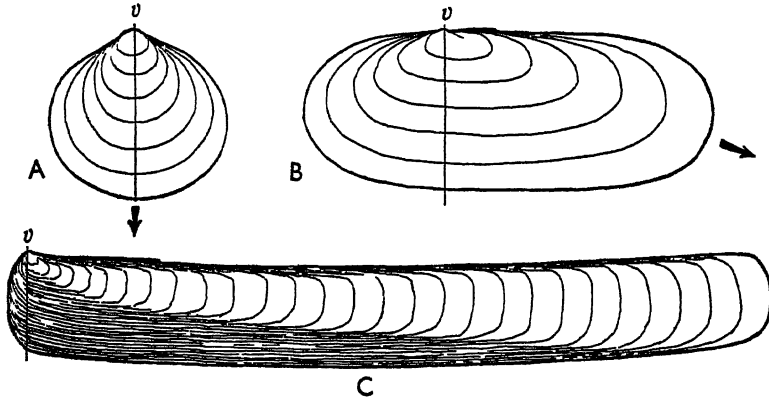


Fig. 6. Shells, viewed from left side of A, *Glycymeris*; B, *Siliqua patula*; C, *Ensis arcuatus*. v, median vertical axis of shell. Arrows indicate direction of greatest marginal increment of shell.

When the region of greatest marginal increase is not mid-ventral the form of the shell is different. It is necessary here to consider only cases in which the shell becomes elliptical, the "transverse" axis being the greater and growth lines taking the form of a system of coaxial ellipses. This may come about in one of two ways. There may be *two* areas of maximum growth, anteroventrally and posteroventrally, symmetrically disposed on either side of the median vertical axis. The umbones remain in the center of the long axis dorsally, the shell being prolonged to approximately the same distance anteriorly and posteriorly. This condition is found in certain Tellinacea, as in *Gari*, but to a greater extent (producing longer shells), as discussed below, in *Solecurtus* and in *Tagelus*.

But an elliptical shell may be formed in which the growth lines are asymmetrical on either side of the vertical axis, with the result that the umbones are not in the middle of the dorsal surface. This happens when either of the two centers of maximum growth becomes greater than the other. The shell may become enlarged anteriorly, as, for instance, it is in some degree in *Donax*, but usually it is the posteroventral margin where greatest growth occurs, and then the shell extends posteriorly. This, as shown in figure 6, B, is what happens in *Siliqua patula*, where about two-thirds of the area of the shell is posterior to the umbones.

These elliptical shells represent "deformations" of the symmetrical condition exemplified by the circular shell of *Glycymeris*. They belong essentially to the class of deformations which D'Arcy Thompson has shown "may be represented by the

use of radial coördinates, in which one set of lines are represented as radiating from a point or 'focus,' while the other set are transformed into circular arcs cutting the radii orthogonally" (1942, pp. 1040-41). He selects leaves as his major examples of deformations involving asymmetrical, or unequal, growth on either side of a median axis, in this case the midrib. He does not mention lamellibranch shells, but this may well have been because he failed fully to realize how in these animals the original anteroposterior growth axis of the contained body is dominated by the manner of growth of the containing mantle lobes and shell valves. Thompson continues by observing that these radial coördinates "will be especially applicable in cases where the growing structure includes a 'node,' or point where growth is absent or at a minimum; and about which node the rate of growth may be assumed to increase symmetrically." In the lamellibranch shell valve the umbo represents such a node.

It is only by the use of radial coördinates that the form of the shell in different bivalves can be related. This does not apply also to the contained body with its anteroposterior axis. But, at the same time, this is so influenced by the containing mantle and shell that attempts to relate form in different bivalves by any system of rectangular coördinates, however modified by curvature or shear, as attempted, for instance, by Graham (1949), are unsatisfactory.

The shell in *Ensis* can be derived from that of *Siliqua* by accentuation of asymmetry. Anterior to the vertical axis, growth is reduced to a minimum, the region of maximum growth being confined to a small area at the posterior end. Instead of about 60 per cent of the shell being posterior to the vertical axis, as in *Siliqua*, probably hardly less than 99 per cent is posterior in *Ensis*. As revealed by the growth lines shown in figure 6, C, successive additions to the shell posteriorly are far greater than they are anteriorly or ventrally; hence the formation of a long and bladelike shell.

Huxley (1932), referring to the form of razor shells, states that "the normal symmetrical gradient of the lamellibranch shell, with growth-centre opposite the hinge, is here completely distorted by the development of a second growth-centre at the morphologically posterior (siphonal) margin of the shell." This second growth center, however, represents displacement posteriorly, and also localization, of the major growth center present in the shell of *Siliqua patula*. It is through a very similar form that the postlarval shells of species of *Ensis*, *Solen*, and *Cultellus* pass. Thus Morse (1919) states that in *Solen* (= *Ensis*) *ensis* "the protoconch is orbicular and the next stage... closely resembles a similar stage in *Machaera* (= *Siliqua*) *costata*." Lebour (1919) has shown this to be true for *Ensis siliqua* and *Cultellus pellucidus*, describing and figuring postlarvae up to 0.72 mm. long in which the foot protrudes anteroventrally through an extensive pedal gape, and siphons extend for a length equal to about one-third that of the shell. These postlarvae are much more like adult *Siliqua* than adult *Ensis* or *Cultellus*.

The most significant structures of the body, namely, mouth and anus and foot with retractors, together with adductors and extent of pedal gape, in the three species being considered are shown in figure 7. Comparison reveals that change in form of the shell has not affected the relation of the hinge line to the long axis of the body. Unlike lamellibranchs in which, as a result of byssal attachment, the anterior

adductor is reduced or lost, the two remain parallel; the effect of change in form of the shell is to pull the body out in length. But this is far from being a simple elongation with accompanying reduction in depth; a profound effect is exerted on the symmetry of the body. It appears best to discuss this by reference to three points: (1) the umbo (U); (2) a point midway in length between the extremities of pedal attachment and in breadth between the dorsal and ventral surfaces (X); (3) the center of the pedal gape (V). These three represent: (1) dorsally, the primitive mid-point in length*; (2) centrally, the base of the foot; (3) ventrally, the *functional mid-ventral point*, so considered because it is the middle of the opening through which the locomotor organ projects to make contact with the substratum.

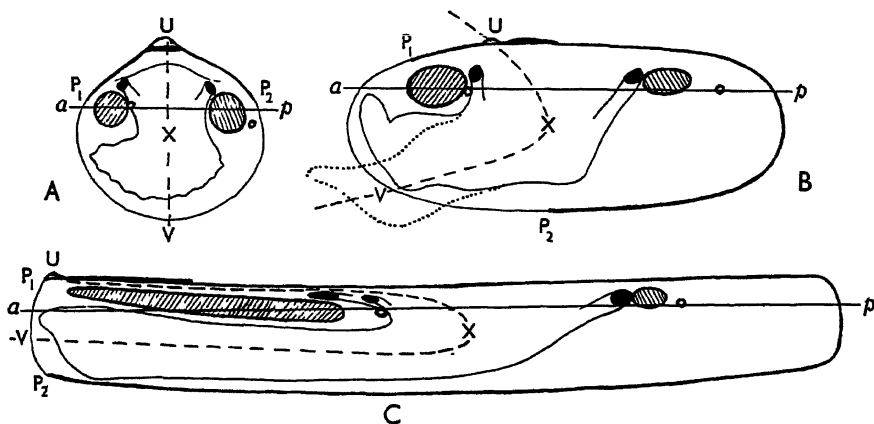


Fig. 7. Diagrams showing effect of shell form on contained animals; adductors, pedal retractors, mouth and anus, and outline of foot alone indicated. *a* -- *p*, anteroposterior axis of body; U -- X -- V, broken line joining these three points (umbo—base of foot—functional mid-ventral point) representing division between anterior and posterior halves of body; *P*₁—*P*₂, extent of pedal gape.

A line joining these three points can be said to divide the animal into anterior and posterior portions. In the former are situated the anterior adductor, anterior pedal retractors, the mouth, and the anterior half of the foot; in the latter, the posterior adductor and retractors, the anus, and the posterior half of the foot. In *Glycymeris* (fig. 7, A) this mid-line is straight and effectively coincides with the vertical and median axis of the mantle and shell. It therefore divides the body into approximately equal anterior and posterior portions. With the loss of the head in the lamellibranchs, owing to enclosure of the body within the mantle, the anterior half of the body has been reduced to the same proportions as the posterior half.

In *Siliqua patula* (fig. 7, B) the mid-line of the body does *not* coincide with the vertical axis of the shell. It is bent, with the two ends pointing anteriorly and the center pulled, as it were, posteriorly, owing to the extension in this direction of the shell. If no factor was concerned other than the displacement of the center of maxi-

* Later work indicates that the mid-dorsal region of the *body* is represented by the middle of the ligament, the umbo being the mid-dorsal point of the *mantle/shell* (Yonge, 1953, in press). The two coincide in the case of *Glycymeris* but not in *Siliqua* or *Ensis* where the broken line in fig. 7 should cut the dorsal surface somewhat to the right (i.e., rear) of the umbo. The general appearance is not, however, significantly different.

um shell growth, then the functional mid-ventral point would be expected to remain as before, i.e., in the vertical axis, directly ventral to the fixed point of the umbo. But the lengthening of the shell is accompanied by anterior and posterior gaping of the valves and by major modifications of the mantle edges involving formation of siphons and extensive ventral fusion. The latter, by reducing the size of the pedal gape, causes the functional mid-ventral point to move forward relative to the umbo.

Further change in shell form leading to the condition found in *Ensis* has effects indicated in figure 7, C. With the umbo as the fixed point, posterior extension of the shell has carried the base of the foot half the length of the shell behind it, and the effectively complete fusion of the ventral margins of the mantle lobes has carried the functional mid-ventral point "forward" to the middle of the anterior margin, actually about halfway along the vertical axis of the shell. The mid-line of the body has now the form of a narrow elongated U within which (see fig. 7, C) are contained the anterior adductor and pedal retractors, the mouth, and the anterior half of the foot. Considered in relation to the vertical axis of the shell all these are "posterior" to the umbo.

Examination of figure 6 will reveal that shell A can be converted into shell B and so to C by distortion of radial coördinates; examination of figure 7 will similarly reveal that the bodies (i.e., omitting the shell, including the umbo) can be similarly converted by distortion of rectangular coördinates. But if the shell and body be considered *together*, then radial coördinates cannot be used; for instance, a radius joining the umbo to the anterior adductor runs in front of the median vertical axis in A and B but behind it in C. When the attempt is made with rectangular coördinates this also fails because a vertical line drawn through the umbo is on one side of the mouth and the anterior adductor in A and B, but is on the other side in C. The form finally assumed in the Solenidae is the result of interaction in growth of the mantle and shell on the one hand and of the body on the other.

The effect of this change in form is to produce a very elongate animal with the functional mid-ventral surface at the anterior end. It is this surface which, whatever the form of animal—and this varies widely, particularly in byssal-attached lamellibranchs—is always in contact with the substratum. It is the normal "under surface." Hence the result of evolutionary change in the Solenidae is to produce bivalves that burrow vertically.

COMPARISON WITH TAGELUS CALIFORNIANUS

The elongated shell of the Solenidae is very like that of certain members of the family Asaphidae (Tellinacea), especially species of *Solecurtus* and *Tagelus*. Like all Tellinacea, these animals are deposit feeders, having characteristic separate siphons, cruciform muscle, modified stomach, and so forth (Yonge, 1949). But the shell is elongate, with dorsal and ventral margins parallel, and the hinge mechanism is not unlike that in the Solenidae. It is therefore not surprising that these genera were formerly included in the Solenidae, as the allied Novaculininae still are by Thiele (1935), but probably erroneously (Yonge, 1949). Apart, however, from significant differences in internal anatomy, these genera differ from the Solenidae in that the umbo remains central. Two growth centers, symmetrically

disposed, one on each side of the median vertical axis, are responsible for elongation of the shell to about the same extent anteriorly and posteriorly.

Tagelus californianus Conrad, the jackknife clam, is the one local representative of such species. It is a southern form (Weymouth, 1920; Keen, 1937), and was not known north of Santa Barbara until it was discovered by MacGinitie (1935) at Elkhorn Slough, where a few specimens were found by the writer in 1949. The general appearance and habits have been described by Weymouth (1920); the disposition of the organs in the mantle cavity and the ciliary currents, by Kellogg (1915). The shell (fig. 8, A) attains lengths of up to four inches and is about four times as long as broad, i.e., somewhat intermediate in form between *Siliqua* and *Ensis*. It gapes at each end, but is more compressed laterally than *Ensis*. The foot is enlarged in much the same manner as in the Solenidae, and *T. californianus* has the same habit of vertical movement but in a permanent burrow, up to sixteen inches deep, in mud. This is described and illustrated by Weymouth (1920).

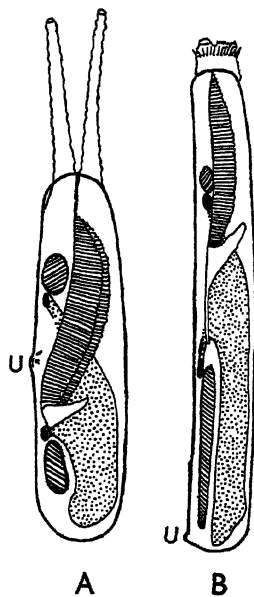


Fig. 8. Comparison between A, *Tagelus*, and B, *Ensis*, showing umboes (U), adductors, pedal retractors, labial palps and ctenidia, foot (stippled), and siphons.

In *Tagelus*, in contrast to *Ensis*, the mid-line of the body coincides with the vertical axis of the shell from the umbo to the center of the foot, but then bends at right angles anteriorly. It is interesting to note that in *Tagelus* the ventral mantle edges are not fused, although they are to a considerable extent in *Solecurtus* and completely in *Novaculina* (Yonge, 1949). But the tubular form of the shell and its position within a permanent burrow excavated in compacted mud restrict pedal movements to the long axis of the shell. The functional mid-ventral point is therefore in the same position as in *Ensis*.

A comparison is made in figure 8 between *T. californianus* and *E. arcuatus*. The fundamental difference between the two resides in their mode of feeding. *T. californianus* is a deposit feeder and this requires long and separate siphons, bottom material being actively taken in through the inhalant siphon, and large ctenidia for creation of powerful inhalant current (Yonge, 1949). Changes in shell growth which tended to draw the base of the foot further within the mantle cavity and thus to reduce the size of both the siphons and the ctenidia (in the Solenidae obviously reduced in *Ensis* when compared with *Siliqua*; see figs. 3 and 4) would not have survival value in the Tellinacea. The Solenidae, however, are suspension feeders, and in them increase in the size of the foot is more important than large siphons or ctenidia. Increased speed of burrowing renders long siphons unnecessary, and reduction in the size of the ctenidia has been shown to be accompanied by increased powers of selection. Moreover, in the exposed and unstable substratum they inhabit, speed of movement is of prime importance. In *T. californianus*, where the foot cannot be quite so large, movement is easier because it takes place in a permanent

burrow. And this in turn is possible because the feeding habits of the animal demand supplies of organic detritus which occur only in still water where mud collects and forms a stable substratum.

The form of the shell can and does vary enormously in the Lamellibranchia, owing to changes in the position and extent of growth centers around the margin. If these be regarded as random variations, then the test of their value is supplied by their effects on the body which the shell encloses. Both *Ensis* and *Tagelus* are elongated and burrow vertically, but in *Ensis* there is one major growth center at the posterior end, and in *Tagelus* there are two, one at each end. In each, the needs of the body, in terms of the habits and habitat, provide evidence why a shell of that particular growth form has been selected.

SUMMARY

Siliqua patula inhabits very exposed beaches. The necessary powers of rapid vertical movement are provided, as in all Solenidae, by the enlarged foot and elongated shell.

Compared with the more specialized *Ensis arcuatus*, *S. patula* has a shorter and broader shell with a strengthening internal rib, relatively long siphons, and a pedal gape which extends from the anterior adductor to the middle of the ventral surface and is fringed with elaborate tentacles borne on the middle lobe of the mantle edge.

The presence of mantle folds enclosing a waste canal, absent in other Solenidae, may be correlated with the entrance of sand particles through the wide pedal gape.

The ctenidia resemble those of other Solenidae, but the nature of the frontal ciliation and the presence of only one oralward current dorsally indicate great selective capacity. In the series *Siliqua*—*Ensis* and *Solen*—*Cultellus*, life within water of decreasing turbidity is correlated with less selective action on the ctenidia. The relation of dorsal to ventral oralward currents is, respectively, 1:2, 3:2, 3:1.

In *Ensis arcuatus* the functional pedal gape is reduced by cuticular fusion, the fourth pallial aperture representing the morphological posterior end. The now anterior pedal gape is flanked by pedal valves formed by enlargements of the inner lobe of the mantle edge. They prevent sand from entering the mantle cavity. The fourth pallial aperture probably provides an additional outlet for water from the mantle cavity when the pedal muscles contract.

In discussing evolution of form in the Lamellibranchia the mantle (and shell) and the body must be considered separately.

The elliptical shell of *Siliqua* and the linear shell of *Ensis* can be derived from a rounded symmetrical shell (e.g., of *Glycymeris*) by displacement of the center of maximum shell growth posteroventrally and then posteriorly. The hinge and umbones become situated at the extreme anterior end, practically all the mantle and shell being posterior to the vertical axis in *Ensis*.

The body retains the anteroposterior growth axis with, owing to loss of the head, anterior and posterior halves of similar size. The form of the body is profoundly modified by that of the mantle and shell. In the Solenidae the mid-line of the body, separating anterior and posterior halves, is consequently bent into a U shape.

The functional mid-ventral point, i.e., where the foot issues to make functional contact with the substratum, becomes anterior, as does the dorsal region associated

Unlike modifications of form due to byssal attachment and consequent heteromyarianism, the anteroposterior axis of the body remains parallel to the dorsal surface of the mantle and shell.

Only by the use of *radial* coördinates can the form of the *shell* in different lamelibranchs be related, but the form of their *bodies* can be related only by the use of *rectangular* coördinates. The form finally assumed is the result of interaction in growth of the mantle and shell on the one hand and of the body on the other.

The form of the shell in the Solenidae is compared with that in *Tagelus* (Tellinacea; family Asaphidae) in which the elongate form is due to two growth centers, anterior and posterior to the median vertical axis. Differences in organization of the body are shown to be associated with differences in feeding, the Solenidae being suspension feeders, and *Tagelus*, with other Tellinacea, a deposit feeder.

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STUDIES ON
PACIFIC COAST MOLLUSKS
V. STRUCTURE AND ADAPTATION IN
ENTODESMA SAXICOLA (BAIRD) AND
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*With a Discussion on Evolution within the Family
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BY
C. M. YONGE

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*With a Discussion on Evolution within the
Family Lyonsiidae (Eulamellibranchia)*

C. M. YONGE
University of Glasgow

INTRODUCTION

THE EULAMELLIBRANCH family Lyonsiidae, included within the suborder Anatinacea by Pelseneer (1906) and in the suborder Anomalodesmata by Thiele (1935), comprises the four genera *Lyonsia*, *Mytilimeria*, *Entodesma*, and *Agriodesma*, the two last being regarded by Thiele as subgenera of *Lyonsia*. Species of *Lyonsia* s.s. are widely distributed: for example, *L. norvegica* off the northwest coast of Europe, *L. hyalina* and *L. arenosa* from the Atlantic seaboard of North America, and *L. californica* and *L. gouldii* from the North Pacific. Information is available about some of these species, all of which burrow in sandy substrata. While possessing characteristic structural features, they do not differ significantly from the majority of other burrowing lamellibranchs.

Interest resides in the peculiarities of form and habit found in the other genera. Evolution within the Lyonsiidae has proceeded in the direction of an increasing tendency toward sedentary life. This is well shown by two species which occur along the Pacific coast of North America. *Entodesma saxicola** (Baird) is found, usually intertidally, in holes or between boulders where it lives embedded in sand, incapable of movement in adult life. *Mytilimeria nuttallii* Conrad lives completely embedded within compound ascidians. There is increasing reduction in calcification and greater irregularity in form in the series *Lyonsia*, *Entodesma*, *Mytilimeria*. The foot is reduced; the byssus is retained into adult life in *Entodesma*, but is lost again in adult *Mytilimeria*. Attention is also arrested by unusual shell characters, notably the extensive union dorsally of the shell valves by periostracum and the presence of a calcareous ossicle or lithodesma which separates the ligament for most of its length.

Examination in life of both these species was undertaken at the Hopkins Marine Station, Pacific Grove, California, during the summer of 1949. Much assistance in collecting was received, particularly from Dr. Cadet Hand and also from Dr. D. P. Abbott, who has since kindly supplied additional preserved material of *Mytilimeria*. Acknowledgments are also due to Mr. G. Owen, B.Sc., who assisted in the preparation of sections in the Department of Zoölogy, University of Glasgow, where this paper has been prepared. Preserved specimens of *Lyonsia norvegica* were obtained from the Marine Station at Millport, Scotland.

* The generic name *Entodesma* is employed to conform with its use by Keep and Baily (1935), Keen (1937), and Smith and Gordon (1948), although Thiele (1935) refers this species to the genus *Agriodesma* Dall.

ENTODESMA SAXICOLA

Habitat and shell.—*Entodesma saxicola* is widely distributed along the Pacific coast of North America between 30° and 54° N latitude (Keen, 1937). At Monterey, California, this species is described by Smith and Gordon (1948) as occurring "Between tides, under boulders and in rock crevices; fairly common." But its habit of embedding itself among particles of sand and shell gravel makes it difficult to find. Specimens were obtained up to 3.5 cm. long and 2.3 cm. broad. Keep and Baily (1935) describe the shell, which is shown in figures 1 and 2. Specimens are seldom as regular as that shown in figure 1, where the basic form is indicated. The shell is

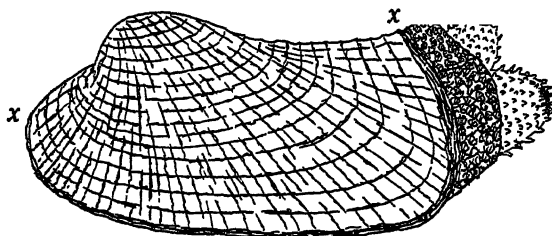


Fig. 1. *Entodesma saxicola*, viewed from left side with siphons extended, basal region of these covered with periostracum with adhering sand grains. $\times 2$. $x-x$, extent of periostracum uniting shell valves dorsally.

elongate, with the umbones, as in all Lyonsiidae, nearer to the anterior than to the posterior end; they are directed anteriorly and diverge from one another. The shell valves are rounded anteriorly, but are somewhat compressed laterally behind.

The calcareous shell, largely of nacreous material, is relatively thin and fractures readily. It is everywhere covered by a very thick and irregularly folded layer of periostracum of which the margins of the valves are exclusively composed. A shell 2 cm. broad was bordered ventrally by periostracum 2 mm. wide, and posteriorly by a layer twice as wide. The shell valves gape at this end and the marginal periostracum bends outward like a funnel. The two shell valves are united for their entire length dorsally by periostracum (see figs. 1, 2, 3, $x-x$). This may be a primitive feature. Description of the ligament (fig. 3, L) and the associated calcareous ossicle (fig. 3, CO) is deferred until later.

Periostracum also covers the base of the siphons and ventral surface of the fused mantle edges. Sand grains adhere tenaciously to it. As shown in figures 1 and 2, this is particularly true of the basal region of the siphons, but much sand may also stick to the ventral periostracum (see fig. 2), and sand grains and shell gravel are often attached to the surface of the shell. It seems probable that the periostracum is secreted in a semifluid state and that sand grains become incorporated at this time. One consequence of the dense accumulation of sand around the base of the siphons is that the posterior gape is effectively filled when the siphons are withdrawn. Incorporation of foreign particles may be a consequence of the unusually thick periostracum which is secreted.

Mantle edge and siphons.—Fusion of the mantle edges ventrally is complete in the sense that both inner and middle lobes are involved, with the two periostracal grooves approximated very closely, as described by Atkins (1937b) in the allied

Thracia villosiuscula. There is complete fusion of tissues. There is a small pedal opening (figs. 2 and 3) and a still smaller fourth pallial aperture (A_4), both of which are bounded internally by mobile lips formed from the inner (muscular) lobe of the mantle edge.

The basal region of the siphons, covered with periostracum and incorporated sand grains, represents a posterior extension of mantle cavity, as in *Solecurtus* (Yonge, 1949). The siphons themselves are separate and actually shorter than the basal region (see figs. 1 and 3). In an animal with a shell 2.7 cm. long, the basal region was 5 mm. long and the siphons only 3 mm. long. In life the latter are orange in color and both are covered with many small tentacles. The inhalant, but not the

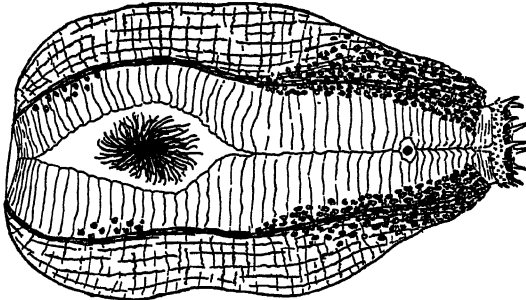


Fig. 2. *Entodesma saxicola*, ventral aspect with animal fully distended, showing byssus threads emerging through pedal gape, also fourth pallial aperture and inhalant siphon. Shell irregular in form, with thick periostracum which also covers ventral surface of mantle edges; sand grains adherent to periostracum. $\times 3$.

exhalant, aperture is fringed with simple tentacles. There is no evidence of any secretion of mucus for the formation of a tube through the substratum, as in the species of *Thracia* (Yonge, 1937).

Organs in the mantle cavity.—The disposition of the organs in the mantle cavity, as revealed after removal of the right shell valve and mantle lobe (apart from its margins) and with the ctenidia intact, is shown in figure 3. The organs in the visceral mass are shown in figure 4, after removal of the right ctenidium. In association with the posterior extension of the mantle and shell, the posterior adductor is the larger, the anterior muscle being displaced ventrally. The general disposition of the major organs is strikingly similar to that in *Sphenia binghami* (Yonge, 1951). This is a member of the Myacea, but is similarly attached by a byssus with which this heteromyarian condition is associated.

The foot is very small and concerned exclusively in adult life with byssus formation; as shown in figure 2, a thick tangle of byssal threads projects through the small pedal opening. There are small anterior and posterior pedal retractors, or, more accurately, byssal muscles.

The labial palps (LP) are small and straplike. The ctenidia expose extensive lateral surfaces because, although the axis runs along the middle of the body (see fig. 4, E), the outer demibranchs are turned up and attached near the dorsal surface (see figs. 3 and 4). As noted by Ridewood (1903), Kellogg (1915), and Atkins (1937a) for this and allied species, this demibranch consists of a direct lamella only. The inner demibranch is broad and is reflected. The lamellae, as noted by the

above-mentioned workers, are very deeply plicate and are heterorhabdic, the plication being continued over the axis. The ctenidia are very muscular; contraction within the axis in particular causes marked reduction in length. There is a small marginal groove along the free edge of the inner demibranch.

Ciliary currents.—The ciliary currents have been described and figured for this species by Kellogg (1915). They are similar to those he describes for *Mytilimeria nuttallii* and *Lyonsia californica* and those described by Atkins (1937a) for *Lyonsia norwegica* and also for species of *Thracia* and *Pandora*. Ridewood (1903) noted

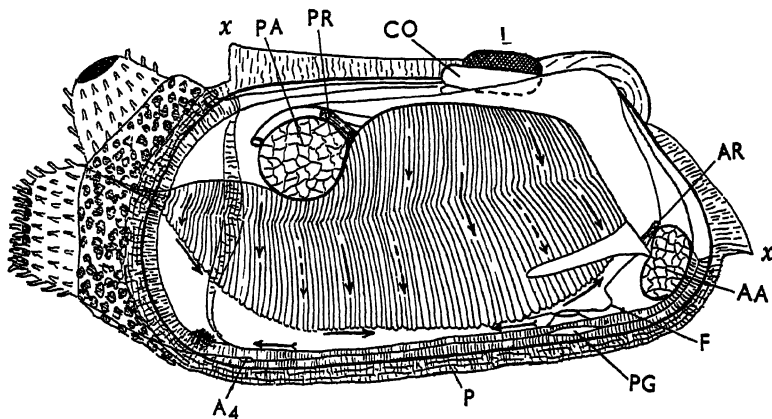


Fig. 3. *Entodesma saxicola*, mantle cavity viewed from right side after removal of right shell valve and mantle lobe. $\times 3$. AA, anterior adductor; AR, anterior pedal retractor; A₄, fourth pallial aperture; CO, calcareous ossicle (lithodesma); F, foot; L, ligament (portion on right of ossicle); P, marginal periostracum; PA, posterior adductor; PG, pedal gape; PR, posterior pedal retractor. x-x, extent of periostracum uniting shell valves dorsally. Plain arrows indicate feeding currents on exposed surfaces; broken arrows, those on undersurfaces; feathered arrows, cleansing (rejection) currents.

the very uniform structure of the ctenidia in these and allied genera of the Anatinacea. On all faces of the ctenidia, frontal cilia carry particles exclusively ventralward where, along the marginal groove alone, there is an oralward current (see arrows in fig. 4). On the surface of the visceral mass and of the mantle, particles are carried toward the mid-ventral region. There, together with material rejected from the palps or from the marginal groove of the inner demibranchs, they are conveyed as usual to the embayment at the base of the inhalant siphon, where masses of pseudofaeces collect and are periodically ejected.

Fourth pallial aperture.—This small rounded opening lies about 0.9 cm. from the posterior end in a specimen having a shell 2.7 cm. long. When open, carmine particles were observed to be drawn in with the current created by the lateral cilia on the ctenidia. The opening can be closed independently of the siphons following sphincter-like closure of the lips formed from the muscular inner lobe of the mantle edge. This action is usually followed by closure of the shell. There is a similar aperture in *Mytilimeria* and *Lyonsia*, as well as in related genera such as *Thracia* and the deep-sea genera *Asthenothaerus* and *Periploma* (Pelseneer, 1911). Consideration of its possible function may suitably be left for the discussion.

Reproductive organs and viscera.—As in the Anatinacea generally, according to Pelseneer (1891, 1906), *E. saxicola* is hermaphrodite, possessing two pairs of

gonads, each with its own duct. The general arrangement of the viscera is shown in figure 4; it closely corresponds to that described by Pelseneer (1891) for *Lynosia norvegica*. The gonads are very large; possibly owing to this, the kidney (K) is displaced posterior to the pericardium (PC) and the digestive diverticula (D) anteriorly and ventrally. The ovary (O) occupies the dorsal region of the visceral mass; below it lies the testis (T), which fills the entire distal region, basal to the foot. The openings of the two gonads (FP, MP) and of the kidney (U) lie very close together in a kind of cloaca between the ctenidial axis above (indicated in fig. 4 by

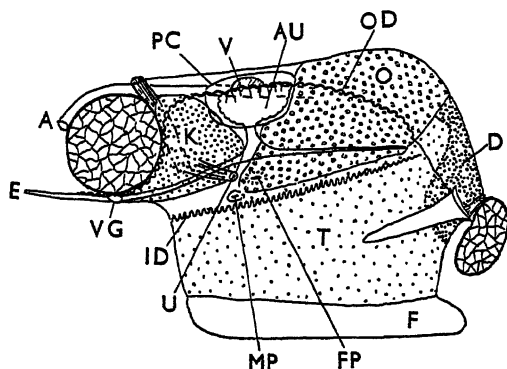


Fig. 4. *Entodesma saxicola*, visceral mass viewed from right side after removal of right ctenidium. $\times 3$. A, anus; AU, right auricle; D, digestive diverticula; E, efferent branchial vessel (runs in ctenidial axis); F, foot; FP, female genital pore; ID, attachment of inner lamella of inner demibranch; K, kidney; MP, male genital pore; O, ovary; OD, attachment of outer demibranch; T, testis; PC, pericardium; U, ureter; V, ventricle; VG, visceral ganglion.

the position of the efferent branchial vessel, E) and the attachment below the inner lamella (ID) of the inner demibranch. This is the region of greatest exhalant flow; hence renal and genital products are carried out immediately through the exhalant siphon.

Both in this species and in *Mytilimeria* there was evidence that the two gonads discharge gametes alternately, a spurt of eggs from the exhalant siphon being followed later by a spurt of spermatozoa, then by eggs, and so on. In both species vast numbers of eggs are spawned; any idea that, on account of the habitat, there might be incubation of larvae in the mantle cavity was soon dispelled. Fertilization and development must occur in the open sea, and larvae must settle in due course from the plankton.

MYTILIMERIA NUTTALLII

Habitat.—*Mytilimeria nuttallii* is stated by Keen (1937) to occur between 20° and 54° N latitude. As noted, among others, by Kellogg (1915) and Keep and Bailly (1935), it lives embedded in compound ascidians. There is thus a resemblance in habit to *Musculus* (*Modiolaria*) *marmoratus* of north European seas, which often, though not always, lives buried in the test of simple ascidians. *M. nuttallii* is particularly abundant in the thick reddish masses of *Eudistoma psammion*, though it does occur also in *Distaplia occidentalis*. Within these colonies it is common between the tide marks, although it is reported to depths of 10 to 20 fathoms off Monterey (Smith and Gordon, 1948). When the siphons are withdrawn, the presence of this

bivalve is revealed by slits, up to 1 cm. long and 1–2 mm. wide, on the surface of the ascidian colonies. Those of *E. psammion* are up to 3 cm. thick, and specimens of *M. nuttallii* are firmly embedded, with the posterior end, which is uppermost, some 4 mm. below the surface when fully grown. The shell seldom extends to the surface of the rock below, and it appears that the bivalve normally settles on the ascidian colony. There is no evidence that the ascidian grows around and over previously settled *Mytilimeria*, although, as noted below, one specimen was found growing apart from ascidians. After settlement of the young mollusk, the growth of the

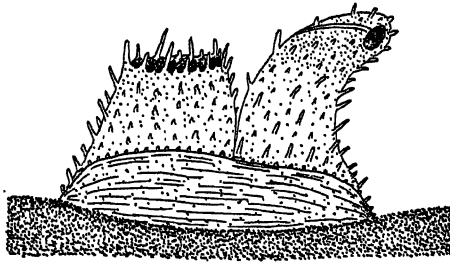


Fig. 5. *Mytilimeria nuttallii*, appearance of fully extended siphons projecting above surface of colony of compound ascidian, *Eudistoma psammion*. $\times 4$.

ascidian below it appears to be inhibited, but the colony continues to grow up on all sides, eventually enclosing it, except for the slit which is kept open by projection of the siphons. The ascidians certainly live much longer than the mollusk, the dead shells of which are frequently found embedded within the colonial mass.

Shell.—Keep and Baily (1935) describe the shell as being "like an inflated bladder with spiral beaks at one end." Such globular shells, up to 2.5 cm. in diameter, do occur where growth is unimpeded, but many are distorted or irregular in shape. This is probably due, in part at any rate, to the very thin shell, which was found to be no more than 0.17 mm. thick in an animal 2.2 cm. long. There is the same great thickness of periostracum as in *Entodesma*, but in *M. nuttallii* it becomes everywhere intimately attached to the substance of the ascidian colony instead of to sand grains. One interesting example was found, however, of an animal which had grown apart from ascidians. The shell was regular, being rounded ventrally, and covered by sand grains, shell fragments, and other debris attached to the periostracum. The shell of this animal was perhaps slightly thicker than usual.

As shown in figure 6, there is the same extensive attachment dorsally of the shell valves by periostracum as in *Entodesma*. The hinge is similar, without teeth and with the unpaired calcareous ossicle (CO) associated with the ligament (L). The umbones show the same features, curling outward anteriorly.

Siphons.—Only the periostracum which covers the fused region at the base of the siphons is free from attachment to the ascidian. As shown in figure 5, this is protruded above the surface of the colony when the valves open and the siphons extend. The latter are very like those of *Entodesma*. Both would seem to belong to siphons of Type A (Yonge, 1948a), which are formed from the inner lobe of the mantle edge. The middle lobe, with the periostracal groove, extends only over the fused basal region. In color the siphons of *M. nuttallii* are opaque white, but are covered with fine pigment spots which give an over-all grayish or reddish appearance. As

shown in figure 5, when fully extended the exhalant siphon is somewhat longer than the inhalant siphon, from which it diverges at the tip.

Organs in the mantle cavity.—Apart from the more rounded shape of the cavity (see fig. 6), conditions are very similar to those in *Entodesma*. With further reduction in the anterior portion of the body, the heteromyarian condition is more marked, with further reduction in the size of the anterior adductor. The foot is smaller and retains a byssal groove. There is evidence in smaller individuals of the byssus threads by means of which the animal must initially be attached but which

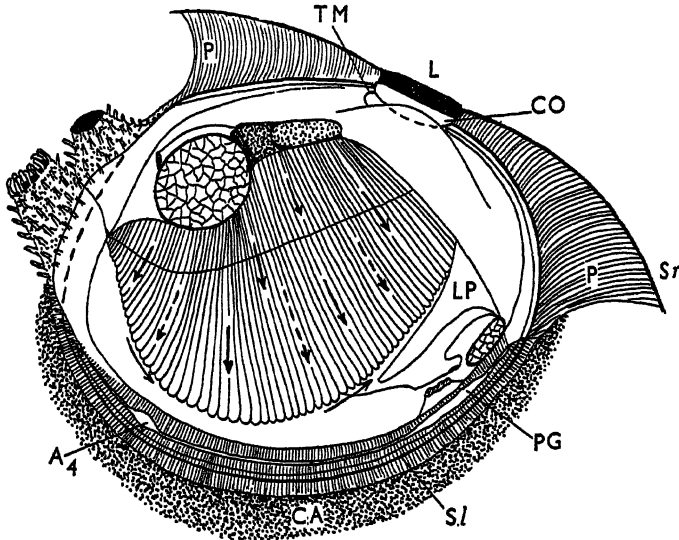


Fig. 6. *Mytilimeria nuttallii*, mantle cavity viewed from right side after removal of right shell valve and mantle lobe. $\times 3\frac{1}{2}$. CA, compound ascidian to which periostracum everywhere adheres; LP, labial palp; P, periostracum uniting shell valves dorsally; SL, SR, margins of left and right shell valves; TM, tongue of mantle which extends forward dorsal to the calcareous ossicle which it secretes. Other lettering, and arrows, as in fig. 3.

are unnecessary when it becomes entirely embedded within the common test of the ascidian colony. In correlation with the loss of function of the foot, both as a means of locomotion and of attachment, the pedal retractors have become extremely small. The pedal aperture remains about the same size as in *Entodesma*, and so does the fourth pallial aperture. The palps and ctenidia are essentially similar.

Ciliary currents.—Kellogg (1915) has described in detail, with a number of figures, the course of currents on the surface of the ctenidia, palps, visceral mass, and mantle. All follow the same general course as in *Entodesma*.

Reproductive organs and viscera.—Again the arrangement of organs is essentially similar to those already described, and the animals are hermaphrodite. Interesting observations were made during the process of spawning. The siphons are then far extended and also dilated. The membrane bordering the exhalant siphon was seen to vibrate with the force of the outgoing current of water. An alternate extrusion of eggs and then of spermatozoa was observed in intact animals. Incubation in the mantle cavity does not occur.

EVOLUTION WITHIN THE LYONSIIDAE

After the foregoing account of species adapted for sedentary life, discussion may suitably be concerned with the manner in which they may have evolved from more active ancestors. These were probably not unlike *Lyonsia*, with which comparison will be made.

Species of *Lyonsia*, although widely distributed, are seldom numerous and appear to be among the least successful of the many lamellibranchs inhabiting soft substrata. Their habits are rather obscure; although certainly not attached, in adult life the foot and particularly the pedal gape (see fig. 8, A) are small. This is also true of species of the allied genus *Thracia*, which can certainly rebury themselves,

though slowly, if removed from the substratum (personal observations). But once buried they remain indefinitely in one position. This habit, which is probably also that of species of *Lyonsia*, is unusual for shallow burrowers.

Pelseneer (1891) has described the viscera, including the hermaphroditic reproductive system, in *Lyonsia norwegica*, and those of *E. saxicola* and *M. nuttalli* are similar. Changes in habit have not been accompanied by changes in mode of development. There are close resemblances between the shells of the three species. Even in *Lyonsia* the shell is fragile and largely nacreous (see Bøggild, 1930); the periostracum is thick and wrinkled and often incorporates sand grains, as described by Morse (1919) in *L. hyalina*. As indicated in figure 8, the valves are always united by periostracum for the wide extent dorsally between the two adductors. This possibly primitive mode of union may well be correlated with the absence of hinge teeth and possibly with the presence, in all species, of the calcareous ossicle or lithodesma which is associated with the ligament. As shown in figure 7, this consists of an elongate plate (CO) which is attached laterally and anteriorly

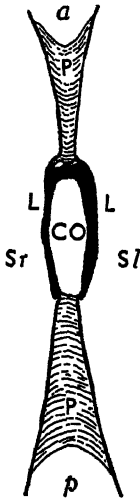


Fig. 7. *Entodesma saxicola*, dorsal region of shell valves viewed from ventral aspect, showing nature of attachment. $\times 2$. a, p, anterior and posterior; CO, calcareous ossicle (lithodesma) attached to ligament (L) laterally and anteriorly, free posteriorly; P, periostracum uniting shell valves (Sl, Sr).

to the ligament (L). It is free posteriorly, and a tonguelike projection of the mantle (fig. 6, TM) extends forward between the periostracum (P) uniting the shell valves (Sl, Sr) above and the calcareous ossicle below. This region of the mantle is concerned with the secretion of both the ossicle ventrally and the ligament. The latter directly unites the two valves for a short distance at the anterior end of the ossicle (see fig. 7), but anterior to that it is separated by the diverging umbones (especially obvious in *Entodesma* and *Mytilimeria*), and posteriorly by the ossicle. No functional significance can be attributed to the peculiar arrangement of the ligament and lithodesma in the Lyonsiidae. A similar condition occurs in *Myodora*, *Myochama*, and *Chamostrea* (Thiele, 1935). Examination of these and of members of related families of the Anomalodesmata is necessary before valid conclusions can

be drawn about the origin and possible functional significance of this peculiar divided ligament.

All species of the Lyonsiidae are suspension feeders. The form of the ctenidium, with reduced and dorsally extending outer demibranchs, is the same in all, and so are the ciliary currents as described by Kellogg (1915), Atkins (1937b), and in this paper. All have a small fourth pallial aperture. Kellogg (1915) was unable to find a function for this. Similar openings in species of the Mactridae are concerned with removal of pseudofaeces (Yonge, 1948b); others in the Solenidae probably act as safety valves, permitting escape of water from the mantle cavity when the enormous foot is suddenly withdrawn (Yonge, 1952). In the Lyonsiidae and in related genera the aperture certainly has neither of these functions, the second of

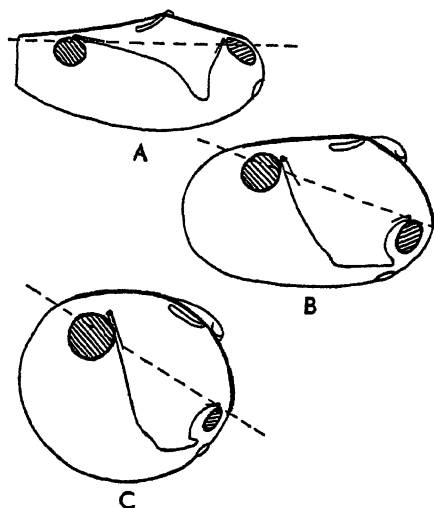


Fig. 8. Lyonsiidae, diagrams showing change in form within family. Figures show adductors, pedal retractors, extent of periostracal attachment and position of calcareous ossicle dorsally, position of pedal gape ventrally. Broken line, drawn joining mouth and anus, indicates antero-posterior axis of body. A, *Lyonsia*, not attached, dimyarian; B, *Entodesma*, byssal attached, ventral migration and reduction of anterior adductor; C, *Mytilimeria*, embedded, markedly heteromyarian but circular outline of mantle and shell.

them clearly unnecessary. The presence of this opening in all three genera, despite the different modes of life, suggests that it may be a purely structural feature, possibly due to the manner in which the mantle edges fuse during development.

The differences between *Entodesma saxicola* and *Mytilimeria nuttallii* on the one hand and *Lyonsia norvegica* (as an example of the genus) on the other are associated with the effects of attachment in the first two. Differences between the two are connected with their different modes of attachment. The major points to be discussed are indicated diagrammatically in figure 8.

In the unattached *Lyonsia* (A), the mantle and shell are extended posteriorly; the ratio of length, anterior and posterior to the umbones, is 1:1.3. The adductors are similar in size, and the anteroposterior axis of the body (drawn from mouth to anus) is roughly parallel to the long axis of the shell. In *Entodesma* (B) the effect of byssal attachment is seen; it is essentially the same as in other widely separated genera which are similarly attached, e.g., *Mytilus*, *Dreissensia*, and *Sphenia*. The

ventral margin of the shell is straight, and both shell and tissues anterior to the umbo are reduced. The anterior/posterior length ratio in *Entodesma* is 1 : 3. The anterior adductor becomes markedly smaller and moves ventrally, accompanied by the mouth. Thus the anteroposterior axis of the body ceases to be parallel to the long axis of the shell (see fig. 8, B).

These are associated with byssal attachment of the body to the substratum. Only in *Arca*, where there is an anterior as well as a posterior inhalant current (Atkins, 1936), is such attachment not accompanied by hypertrophy of the posterior regions associated with intake and expulsion of water needed for both feeding and respiration. In unattached lamellibranchs, such as the Solenidae, the form of the body is profoundly influenced by changes in the growth centers of the mantle and shell (Yonge, 1952). But both the adductors remain well developed and the anteroposterior axis of the body continues to be parallel to the long axis of the shell. Precisely the opposite occurs in byssally attached bivalves such as *Entodesma*. There is a great reduction in the anterior half of the body with accompanying reduction of the anterior adductor. The animal becomes heteromyarian and the long axes of the body and of the shell are no longer parallel.

It may be further noted that, in the Filibranchia, continued byssal attachment has led eventually to loss of the anterior adductor, i.e., to byssally attached monomyarians such as *Pinctada* and *Avicula*, followed in some genera by secondary freedom, as in *Chlamys* and *Pecten*, and then again by renewed attachment, this time by cementation, as in *Spondylus* and *Hinnites* (Yonge, 1951b). In the Tridacnidae, byssal attachment has also led to monomyarianism but by a different route (Yonge, 1936). The monomyarian condition in the Lamellibranchia is fully discussed elsewhere (Yonge, 1953, in press).

In the Lyonsiidae, byssal attachment resulting in the heteromyarian condition has, very interestingly, been followed by life embedded within a mass of compound ascidians. The assumption of this habit may reasonably be attributed first to byssal attachment and then to the adhesive properties of the periostracum, present in *Lyonsia*, pronounced in *Entodesma*, and in *Mytilimeria* enabling the shell to unite intimately with the surrounding ascidian mass. But once embedded within this, the initial byssal attachment is lost. In the absence of this localized constraint the mantle and shell assume a rounded form. They do so also, it may be noted, in cemented lamellibranchs no matter whether these are monomyarian, e.g., *Ostrea* or *Spondylus*, or dimyarian, e.g., *Chama*. But *Mytilimeria* appears to be a unique instance of the heteromyarian condition associated with a rounded shell, as shown in fig. 8, C. This comes about because byssal attachment has been replaced by embedding in ascidians. The animal settles upon a *living substratum* which grows upward and around it.

SUMMARY

Within the Lyonsiidae there is an increasing tendency toward sedentary life. *Lyonsia norwegica* burrows in sandy substrata; *Entodesma saxicola* is attached by byssus in rock crevices; *Mytilimeria nuttallii* is embedded within compound ascidians.

The habits, major structural features, and ciliary currents of the two last are described and compared with those of *Lyonsia*.

In all, the calcareous shell is thin and largely nacreous, but the periostracum is very thick and adheres to foreign matter. The hinge is without teeth, the ligament is divided by a central calcareous ossicle or lithodesma, and dorsally the shell valves are widely united by periostracum.

The tissues of the mantle edge are extensively fused, but all have a small fourth pallial aperture as well as a small pedal gape. The former has no obvious function and may be a purely structural feature.

The structure of the ctenidia and the nature of the ciliary currents on these are the same in all three species; the frontal cilia everywhere beat ventralward, and the only oralward current is along the free margin of the inner demibranch.

All are hermaphrodite with separate gonads, each having its own duct. Eggs and sperm are liberated alternately; there is no incubation of larvae in the mantle cavity.

The differences in the Lyonsiidae are in the form of the shell. As a result of byssal attachment, the anterior portions of the body and mantle (with shell) in *E. saxicola* are reduced. The animal becomes heteromyarian, as do many unrelated but similarly attached species.

In the Filibranchia, further effects of such attachment have been the loss of the anterior adductor and the appearance of monomyarians such as *Pinctada*—later of free and then of some cemented genera. In the Lyonsiidae further evolution has occurred in *Mytilimeria*, which settles on the living substratum of ascidian colonies.

Within the ascidian, local constraint due to byssal attachment is lost and the animal assumes a rounded form which is unusual, if not unique, in a heteromyarian.

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A NOTE ON KELLIA LAPEROUSII (DESHAYES)

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THE LAMELLIBRANCHIA associated by Pelseneer (1911) within the Leptonacea and by Thiele (1935) within the Erycinacea are of unusual interest. They consist of animals initially adapted for movement on a hard substratum, almost like gastropods, and with an anterior inhalant opening, sometimes prolonged into a solitary siphonal tube. The ctenidia are frequently reduced. There is a further tendency toward sedentary existence through attachment by a few byssal threads. This has led to many varied and interesting cases of commensalism with decapod and stomatopod Crustacea, with ophiuroid, synaptid, and echinoid Echinodermata, with corals, and with annelids and sipunculids (for further account and references see Leyborne Popham, 1939, 1940; also Smith and Gordon, 1948). The process culminates in the one known instance of endoparasitism in the Lamellibranchia, namely, *Entovalva mirabilis*, which lives in the gut of a holothurian (Voeltzkow, 1891).

But even in the free-living species there is much structural modification. Thus in *Galeomma turtoni* the shell valves gape ventrally, the wide space being bridged by fusion of the enlarged inner lobes of the mantle edge. The animal moves on rocks by means of the foot, which has a creeping sole. In *Chlamydoconcha orcutti* Dall, which occurs along the southern half of the coast of California, the shell is very greatly reduced and entirely enclosed within the mantle. Unfortunately no specimens of this species were obtained; it is reported as rare at Monterey, California (Smith and Gordon, 1948), but the process of overgrowth is probably the same as in *Phlyctaenachlamys lysiosquillina*, commensal in the burrows of *Lysiosquilla maculata* along the northeast coast of Australia. Here, as described by Leyborne Popham (1939), the middle lobe of the mantle edge grows around and finally completely encloses the much reduced shell.

The least modified genus within the Erycinacea would seem to be *Kellia*, at any rate judging from what is known of the common species, *K. suborbicularis* (Montagu). This occurs in European waters and also along the Pacific coast of North America, where, according to Keen (1937), it has an exceptionally wide range, between 8° and 56°. This species is well known, extending from the shore into the sublittoral zone and is typically attached by a few byssal threads in holes in stones or within dead shells. The life history has been described by Lebour (1938); the young are incubated, for a limited time only, in the mantle cavity. The appearance of the animal in life is described and figured by Leyborne Popham (1940), who also described the arrangement of feeding currents and rejection currents on the ctenidia and on the surface of the visceral mass and mantle. When expanded, the mantle edges project for only a short distance beyond the margin of the shell valves, and the siphonal openings are short also. These statements are confirmed by personal observations.

Along the coast of California, although over a more restricted range, from 30° to 60° (Keen, 1937), occurs a second species, *K. laperousii*. This is described by Keep and Bailly (1935) as larger than *K. suborbicularis*, darker in color and more

oval in shape, and as living in the shelter of holes made by piddocks and other borers. Smith and Gordon (1948) suggest, however, that since it occurs with *K. suborbicularis* it should possibly be included within that species. One purpose of this note is to show that *K. laperousii* is undoubtedly a distinct species.

A number of examples of this animal were obtained between tide marks, some at Frenchman's Reef near San Francisco, others around Pacific Grove. All were

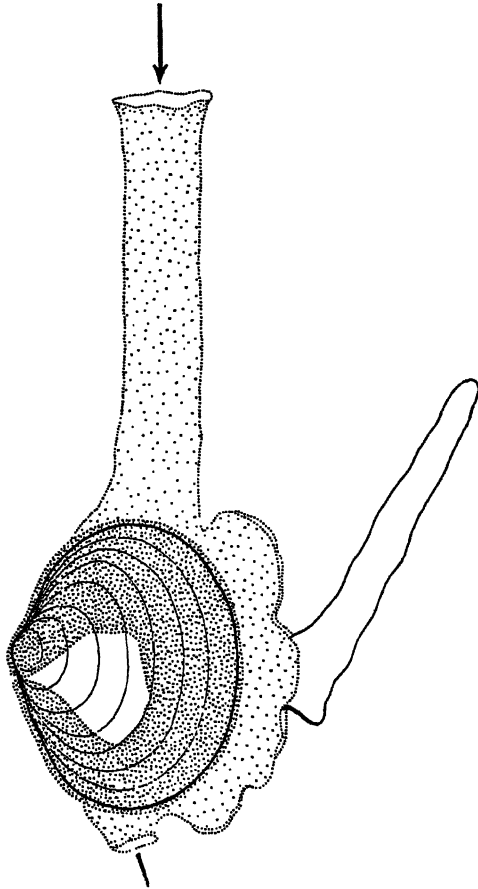


Fig. 1. *Kellia laperousii*, viewed from right side with mantle edges and foot fully distended. $\times 2$. Middle lobe of mantle edge (darker stippling) spreading over surface of shell valve, inner lobe (lighter stippling) forming long inhalant (anterior) and short exhalant (posterior) siphons, also margins of pedal gape. Arrows indicate inhalant and exhalant currents.

found inhabiting relatively deep bore holes in rock, as stated by Keep and Bailly, or the cavities of empty mussel shells, and the like. The largest specimen collected had a shell 2.0 cm. long, 1.6 cm. broad, and 1.1 cm. thick. These measurements are greater than any recorded for *K. suborbicularis*, although the shell in that species is more globular. Many examples of *K. suborbicularis* were found also.

K. laperousii was usually found attached by up to four fine byssal threads, but when freed could move about with the aid of the long, thin foot (see fig. 1). This has a creeping sole and a byssal gland which is situated in the heel.

The striking feature about these animals, and one seen first when they were encountered on the shore, is the great extension of the mantle edges beyond the margins of the shell valves. As shown in figure 1, the middle lobes of the mantle edge (more darkly stippled) extend around the margin of the shell valves and over the greater part of the outer surface of these. The inner mantle lobes (lightly stippled) protrude anteriorly and posteriorly to form the inhalant and exhalant siphons (indicated by arrows), and also ventrally where they bound the wide pedal gape. The inhalant siphon is of great length, up to twice that of the shell. All these tissues can be withdrawn completely.

The striking elongation of the inhalant siphon is of great interest, and would seem to be unique among nonburrowing lamellibranchs. It appears to be correlated with the habitat, in the protection of deep bore holes and crevices of various kinds. This long siphon enables the animal to draw in clear water and to avoid the debris that inevitably collects within the cavities it occupies. *K. laperousii* differs, of course, from deep burrowers with long siphons in that the inhalant siphon is *anterior* and it alone is lengthened.

Several of the shells were much eroded around the umbones. The cause of this is obscure—possibly the poor development of protective periostracum or else the result of violent agitation within cavities in the surf zone. But the extension of the middle lobe of the mantle edge appears to protect the rest of the surface of the shell valves, which are not eroded.

The interest of these observations may be briefly listed:

1. The separation of *K. laperousii* from *K. suborbicularis*, in addition to less definite shell characters, by the much greater protrusion of the mantle edges in the former, with the formation of a conspicuously long inhalant siphon.
2. The adaptation, represented by this extension of the inner and middle lobes of the mantle edge, to a nestling habit in relatively deep cavities. This may well be of major advantage to an animal living on shores which are subjected to the heavy surf of the open Pacific.
3. The discovery that in a species of *Kellia*, as well as in the much more specialized *Chlamydoconcha* and *Phlyctaenachlamys*, the middle mantle lobe extends, when the animal is relaxed, over much of the outer surface of the shell valves.

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